Effect of Drought on Carbon Cycling in Model Temperate Grassland and Heathland Plant-Soil Systems

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Zusammenfassung

Es wird erwartet, dass sich Wetterextreme in Europa in den nächsten Jahren häufen werden, besonders während der Hauptwachstumsphase der Pflanzen (April-September). Vor allem extrem lange Trockenphasen können sich negativ auf Graslandökosysteme in Europa auswirken. Diese Ökosysteme machen fast 15% der Landoberfläche Europas aus und speichern grosse Mengen an Kohlenstoff (C) im Boden. Die Menge an C, die gespeichert werden kann, hängt von der C-Aufnahme der Pflanzen ab, der Verwertung innerhalb der Pflanzen und dem anschliessenden Transfer in den Boden. Längere Phasen von Trockenheit können das Pflanzenwachstum vermindern und den C-Gehalt im Boden durch erhöhte Pflanzenmortalität und den Abbau der organischen Bodensubstanz durch Mikroorganismen verringern. Die Wechselwirkungen zwischen oberirdischen und unterirdischen C-Translokationsprozessen spielen eine kritische Rolle im C-Kreislauf. Diese beinhalten sowohl physikalische als auch chemische und biologische Abbaumechanismen. Bisherige Untersuchungen in diesem Themenfeld werden kontrovers diskutiert und haben noch zu keinem Konsens bezüglich der Mechanismen, die durch lange Trockenphasen beeinflusst werden, geführt. Es ist daher unklar, inwiefern eine extreme Trockenphase im Sommer diese komplexen Prozesse beeinflusst. Neue Erkenntnisse sind erforderlich, um den C-Kreislauf im Boden-Pflanze System während verschiedener Phasen der Trockenheit verstehen zu können. Gleiches gilt für die Normalisierung des C-Kreislaufs nach einer solchen Trockenperiode.

Diese Arbeit basiert auf einem Feldexperiment und verfolgt drei Ziele. Das erste Ziel (Manuskript I) war es, die Langzeiteffekte von wiederholten, jährlichen Trockenphasen (100–1000 Jahr Extreme) von 32–42 Tagen pro Jahr während fünf Jahren zu untersuchen. Im Fokus lagen C- und Stickstoff- (N-) Dynamik und die Isotopen- und Lipidzusammensetzung (*n*-Alkane) in zwei Modell-Ökosystemen (Grasland- und Heideland). Das zweite Ziel (Manuskript II) war es, den Einfluss der drei Phasen einer extremen Trockenheit über 104 Tage (Trockenphase I, Tage 0–40, Phase II, Tage 40–70 und Phase III, Tage 70–104) auf die Lipidzusammensetzung zu untersuchen. Die Analyse von stabilen C-Isotopen und *n*-Alkan-Biomarkern im System Pflanze-Boden lieferte die Möglichkeit, die Verwertung von C in der Pflanze auf der Molekülebene zu verfolgen. Das dritte Ziel (Manuskript III) dieser Arbeit war es, den Einfluss einer extremen Trockenheit auf den C-Kreislauf im System Pflanze-Boden mithilfe eines dreifachen $^{13}CO_2$ Pulse-Chase Markierungsexperiments während verschiedener Phasen einer schweren Trockenheit zu ermitteln. Zusätzlich wurde mithilfe von Bewässerungen in den beiden Modell-Ökosystemen (Grasland und Heideland) untersucht, ob schwere Trockenheiten eine schnelle Erholung des Ökosystems verhindern oder nicht.

Es wurde erwartet, dass durch mehrere Trockenperioden mehr Lipide im Pflanze-Boden

System beobachtet werden können. Entgegen der Erwartung konnten keine Langzeiteffekte auf die Lipiddynamik oder den C-Haushalt festgestellt werden. Dies kann auf eine rasche Erholung des Ökosystems zurückgeführt werden, unter der Voraussetzung, dass die Trockenperiode nicht zu intensiv war (Manuskript I).

Die simulierte Trockenheit beeinflusste die Zusammensetzung der extrahierbaren Lipide und *n*-Alkane in der Sprossbiomasse, in den Wurzeln und im Boden während der ersten Trockenphase signifikant, stagnierte aber mit fortschreitender Dauer. Die stabile Isotopenzusammensetzung (δ^{13}C) des organischen C wies eine Anreicherung (1–3‰) in der Sprossbiomasse, in den Wurzeln und im Boden auf. Die substanzspezifische Isotopie (δ^{13}C) der *n*-Alkane zeigte während der ersten 40 Tage der Trockenheit eine Anreicherung (2–3‰) in der Sprossbiomasse, welche auf eine metabolisch veränderte C-Nutzung der Pflanzen während einer Trockenheit schliessen lässt (Manuskript II).

Während der ersten 40 Tage der extremen Trockenheit erhölte sich das C:N Verhältnis und die δ^{13}C Werte in der Sprossbiomasse, den Wurzeln und im Boden in beiden Modellökosystemen. Der erste $^{13}CO_2$-Puls (Trockenphase I) führte zur höchsten ^{13}C-Tracer Aufnahme in der Sprossbiomasse und nahm in Richtung der Wurzeln und des Bodens ab. Mit dem zweiten $^{13}CO_2$-Puls (Trockenphase II) kam es in allen Pflanzen zu einer geringeren Aufnahme von ^{13}C verglichen mit der ersten Pulsmarkierung. Es wurde keine signifikante ^{13}C-Aufnahme nach der dritten Pulsmarkierung beobachtet. Die ^{13}C-Translokation nahm in Richtung der Wurzeln und dem Boden mit zunehmender Dauer der Trockenheit ab. Interessanterweise zeigte sich selbst nach einer extreme Trockenheit ein rasches Austreiben der Pflanzen und eine vollständige Wiederherstellung des C-Kreislaufs im Boden-Pflanze System während der ersten 10–15 Tage nach Bewässerung (Manuskript III).

Diese Studie konnte zeigen, dass während einer extremen Trockenheit die C-Aufnahme von Pflanzen und die C-Verlagerung in den Boden signifikant reduziert war. Auf molekularer Ebene deutet die Veränderung der Lipidzusammensetzung in Sprossbiomasse und Wurzeln auf die Akklimatisierung der Pflanzen während der initialen Phase der Trockenperiode hin. Die verhältnismässig erhöhte Anreicherung von langkettigen *n*-Alkanen im Boden während der initialen Phase der Trockenheit unterstützt die Annahme der selektiven Erhaltung von langkettigen, Komponenten mit geringer Abbaubarkeit. Während der Trockenphasen II und III gab es keine signifikanten Veränderungen der C- und Lipiddynamik im Pflanzen-Boden System, was auf die Widerstandsfähigkeit der Modellökosysteme hinweist.

Dies ist die erste Studie, in der die C- und Lipiddynamik in einem Pflanze-Boden System während einer extremen Trockenphase detailliert untersucht wurde. Die ermittelten Daten konnten im Hinblick auf Regulierungsmechanismen der Pflanze in Kombination mit den oberirdischen und unterirdischen biogeochemischen Prozessen im Rahmen von lang anhaltenden Trockenperioden zu einem deutlich verbesserten Prozessverständnis beitragen. Allgemein zeigte die Studie, dass temperierte Ökosysteme in Hinblick auf den C-Kreislauf im Boden-Pflanze System auch sehr lange Trockenphasen widerstehen können. Insofern konnte diese Studie darauf hindeuten, dass prognostizierte Klimaveränderungen keinen signifikanten Langzeiteffekt auf die Lipiddynamik und den C-Haushalt im Boden-Pflanze System in temperierten Ökosystemen haben.

Summary

Extreme weather events such as severe droughts are expected to occur more frequently in Europe especially during the most active season of plant growth (April-September). Extremely long drought periods can have a negative impact on grassland ecosystems in Europe. Currently, European temperate grassland ecosystems, which occupy nearly 15% of the total land surface of Europe, are facing a greater temperature and precipitation fluctuation on a seasonal basis. These ecosystems store a high amount of carbon (C) in the soil, which strongly depends on the pattern of C uptake by plants, its utilization within plants and its subsequent transfer into the soil. Droughts can reduce plant growth and decrease C concentration in the soil due to an increase in plant mortality and a decreased decomposition rate of the soil organic matter by soil microorganisms. Here, the relationship between above- and belowground C translocation processes play a crucial role for the C cycle in the plant-soil system that include physical, chemical and biological degradation mechanisms. The available studies on the effect of drought on C cycling in the plant-soil system revealed that results are often controversial, leading to no consensus about the underlying mechanisms controlling the relevant processes. It remains unclear, how an extreme summer drought event will influence these complex processes. In this context, more knowledge is required to understand the C cycling in the plant-soil system under different phases of drought and the recovery of C cycling in the plant-soil system after the drought.

The current thesis is focused on a field experiment (EVENT I) and pursued three goals. The first aim (Manuscript I) was to examine the long-term influence of five years of repeated annual drought (100–1000 extreme, 32–42 days/year) on C, N dynamics, its isotopic and lipid composition (n-alkane) in a model grassland and heathland ecosystem. The second aim (Manuscript II) was to study the influence of three phases of a severe drought for 104 days (phase I from days 0–40, phase II days 40–70 and phase III days 70–104) on the plant and soil lipid composition. The stable C isotope analysis of bulk organic C and n-alkane biomarker in the plant-soil system provided the opportunity to trace the utilization of plant C at a molecular level. The third aim (Manuscript III) of this thesis was to understand the impact of a severe drought on the C cycle in the plant-soil system using a triple $^{13}CO_2$ pulse-chase labelling experiment during three different phases of a severe drought. Additionally, the third aim was to examine the effect of irrigation on a model grassland and heathland ecosystem that previously experienced a severe drought.

It was expected to observe higher lipid concentration in the investigated plant-soil system under drought but contrary to the expectation, no long-term influence of the five years of repeated annual drought was observed in terms of lipid dynamics and C budget. This can be attributed to a rapid recovery of the ecosystem if the applied drought period is not too strong (Manuscript I).

Experimental severe drought significantly affected the total extractable lipid concentration and *n*-alkane chain length especially during the drought phase I in shoots, roots and soil. Stable isotopic composition (δ^{13}C) of bulk organic C showed ca. 1–3‰ enrichment in shoots, roots and soil. The compound specific isotope (δ^{13}C) of *n*-alkanes revealed 2–3‰ enrichment in shoots within the first 40 days of the drought, which can be attributed to metabolic change in C utilization under drought conditions (Manuscript II).

During the first 40 days of a severe drought increased C:N ratios and δ^{13}C values for shoots, root and soil were observed in both investigated model ecosystems. The first $^{13}CO_2$ pulse (drought phase I) was followed by the highest ^{13}C tracer uptake in shoot biomass and decreased towards root biomass and soil. After the second pulse (drought phase II, 40–70 days), all shoots showed lower ^{13}C uptake compared to the first pulse labelling. No further ^{13}C uptake was observed after the third pulse labelling (drought phase III). Similar to ^{13}C uptake by shoots, ^{13}C tracer translocation became lower for roots and soil with increased drought duration. Interestingly, even after a severe drought, when no further C cycling in the plant-soil system took place, a fast re-sprouting and renewed C cycling re-surged in the plant-soil system just within 10–15 days after irrigation (Manuscript III).

Overall, this study could show for the first time a strong impact of a severe drought on plant C uptake, assimilation and belowground C allocation into the soil. At a molecular level, the modification of lipid composition in shoots and roots could be attributed to the acclimatization of plants during the drought phase I. Furthermore, the incorporation of higher proportion of long-chain *n*-alkanes into the soil during the initial drought phase supported the assumption of selective preservation of compounds with comparatively low degradability. During drought phases II and III, no significant change in the plant-soil system indicated that the model ecosystems could resist a severe drought in terms of their C and lipid dynamics.

This is the first long-term field experiment, where C and lipid dynamics were studied in detail in the plant-soil system. The obtained data from the study of the impact of severe drought on C cycling at a molecular level are of great importance. It improves our understanding of the regulatory mechanisms of the plant C uptake and assimilation in combination with the aboveground and belowground biogeochemical processes, if the ecosystems experience an unprecedented drought phase. In general, this study shows that temperate ecosystems could resist to extended drought periods in terms of C cycling in the plant-soil system. Hence, the projected climate change might have no significant impact on the long-term lipid dynamics and C-budget in the plant-soil system of temperate ecosystems.

Contents

Abbreviations

$\delta^{13}C$	Stable isotope composition ($^{13}C/^{12}C$) relative to V-PDB standard in permil (‰)
a.s.l.	Above sea level
ACL	Average chain length
ANOVA	Analysis of variance
C	Carbon
C_{org}	Organic C
CO_2	Carbon dioxide
CPI	Carbon preference index
CSIA	Compound-specific isotope analysis
d.w.	Dry weight
DCM/MeOH	Dichloromethane/methanol
GC-FID	Gas chromatography with a flame ionization detector
GC-irmMS	Gas chromatography coupled to an isotope ratio mass spectrometer
GC-MS	Gas chromatography mass spectrometer
N	Nitrogen
p	Probability
R	Abundance ratio of heavier to lighter isotope
TLE	Total lipid extract
v/v	Volume/volume concentration
V–PDB	Vienna–Pee Dee Belemnite

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Part A

Synopsis

This PhD thesis consists of three different studies conducted on an experiment simulating severe drought followed by irrigation. In part A of the thesis, the main findings of the three individual studies (Manuscript I, II and III) are summarized and the extended outlook and orientation for further research is given. Detailed information about the results and discussions are presented in part B with respect to the individual studies. Manuscript I and III are published, whereas Manuscript II is submitted to an international peer-reviewed journal.

1 Introduction

1.1 Climate change and drought

Drought has emerged as a subject of compendious scientific study in the context of global climate change. Due to climate change, rising mean air temperature accompanied by a reduction in precipitation patterns leads to an increased risk of drought (Ciais *et al.*, 2014). Focussing only on the history of European summer droughts, the assessment by Spinoni *et al.* (2015b) revealed that droughts have been occurring more frequently and repeatedly with varied frequencies, magnitude and duration in the European countries. Apparently, drought conditions have become a recurrent feature of the European climate (Dai, 2011; Spinoni *et al.*, 2015b). The more recent droughts in the last decade as shown in Figure 1.1 have occurred frequently in Europe (Spinoni *et al.*, 2013, 2015a). Central Europe was affected by disastrous drought associated with the 2003 summer heat waves. Furthermore, drought events occurred repeatedly in Central, Eastern, Western and Southern Europe. It is expected to experience even more frequent and extended periods of summer drought in Europe by 2100 (Barriopedro *et al.*, 2011; Dai, 2011).

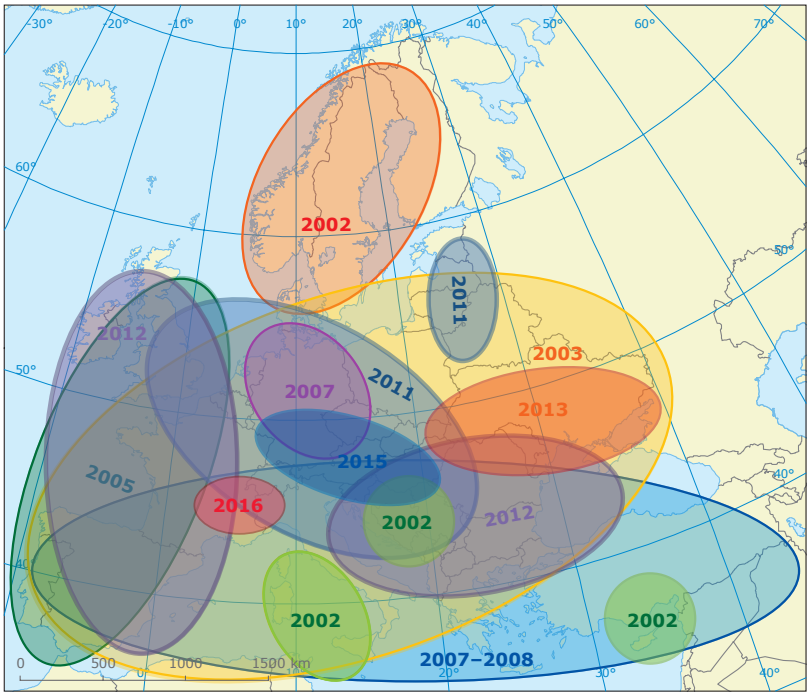

Figure 1.1: Main drought events in Europe. Repeated occurrence of drought events from 2002–2016. Intensity and severity of drought is not shown in this picture (modified after European Environment Agency, 2012).

Each single drought event depends on the fluctuations in the average amount of precipitation and temperature patterns that vary spatially. Therefore, drought conditions need to be defined by the regional/local climate based analysis (Heim, 2002). However, according to Wilhite & Glantz (1985), a conceptual drought can be defined as a prolonged period of abnormally low precipitation combined with increase in temperature. On the other hand, the operational definition of drought was regarded as the onset, severity and termination of the drought (Wilhite & Glantz, 1985).

Drought events can be further classified (Wilhite & Glantz, 1985; Mishra & Singh, 2010) as (a) meteorological drought, which refers to a deficit of precipitation and often accompanied with abnormally high temperature. Since the atmospheric conditions that result in the deficiency of precipitation are highly specific for different regions therefore, the meteorological drought must be based on a specific region. (b) Agricultural drought is defined as a deficit of soil moisture caused by below-average precipitation and above-normal evapotranspiration. Agricultural drought mainly account for the susceptibility of crops during the different stages of the crop development. For example, deficiency in the top soil moisture at planting stage may hinder seed germination and if the deficiency in the top and sub soil moisture continues, a substantial reduction of yield would increase. (c) Hydrological drought develops when stream flow, lake and groundwater levels fall below long-term mean level. Hydrological drought usually occur sometime after the meteorological drought. For instance, first precipitation decreases and some time after that the water level of rivers and lakes decrease. (d) Socio-economical drought is related to the supply and demand of some economic goods. Socioeconomic drought occurs when the demand for an economic good exceeds supply as a result of a weather-related shortfall in water supply.

The above mentioned categories of drought are interconnected to each other and collectively influence on agricultural, ecological and socio-economical stability (Stahl *et al.*, 2016). This thesis mainly focusses on the meteorological drought events that occur in Central Europe.

1.2 Drought impacts

1.2.1 Terrestrial ecosystem

Within the terrestrial biosphere, grasslands cover around 40% of the global land surface area and around 15% of the European total land surface (Carlier *et al.*, 2009; Ciais *et al.*, 2011). Figure 1.2 demonstrates the geographical distribution of extensive and intensive grassland ecosystems in the European Union (Overmars *et al.*, 2014). Extensive grasslands are also known as semi-natural grassland ecosystem. Intensive grasslands are a permanently managed grassland ecosystems (Overmars *et al.*, 2014).

If a drought occurs in consecutive years, especially during the plant-growing season i.e. April - September (Lindborg, 2007) as predicted by the future drought scenario for Central and Mediterranean Europe (Dai, 2011), the ecosystem functions of grasslands will be at a high risk. For example, the summer drought is considered as a primary limitation to the plant productivity, qualitatively and qualitatively (Lei *et al.*, 2016; de Vries & Bardgett, 2016) due to a reduction in the soil moisture. The consequences of decreased soil moisture are directly related to the productivity of the ecosystems.

Soil moisture is controlled by several factors that include primarily soil texture, soil aggregation, organic matter content, soil pH and water holding capacity. Frequent occurrence of drought periods have already affected soil moisture and moreover, the predicted future drought scenario for Europe may have the potential to negatively influence the soil moisture as indicated in Figure 1.3 (European Environment Agency, 2015).

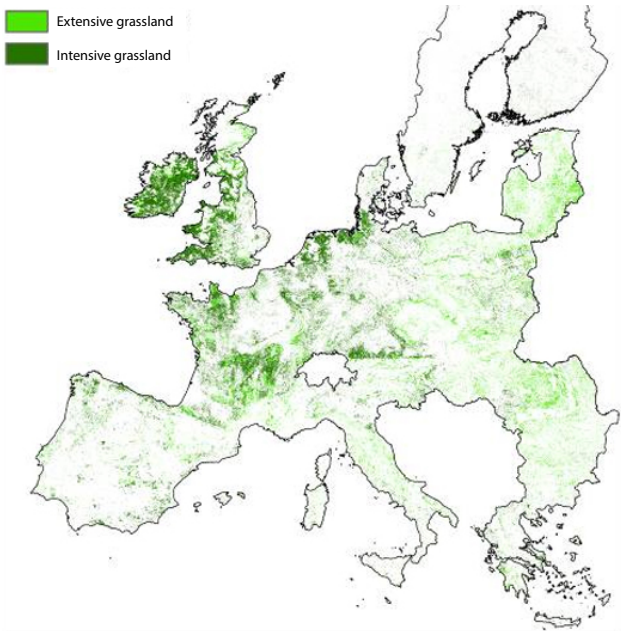

Figure 1.2: Grassland ecosystems of Europe. Geographical distribution of extensive and intensive grassland ecosystems in the European Union (adopted from Overmars *et al.*, 2014).

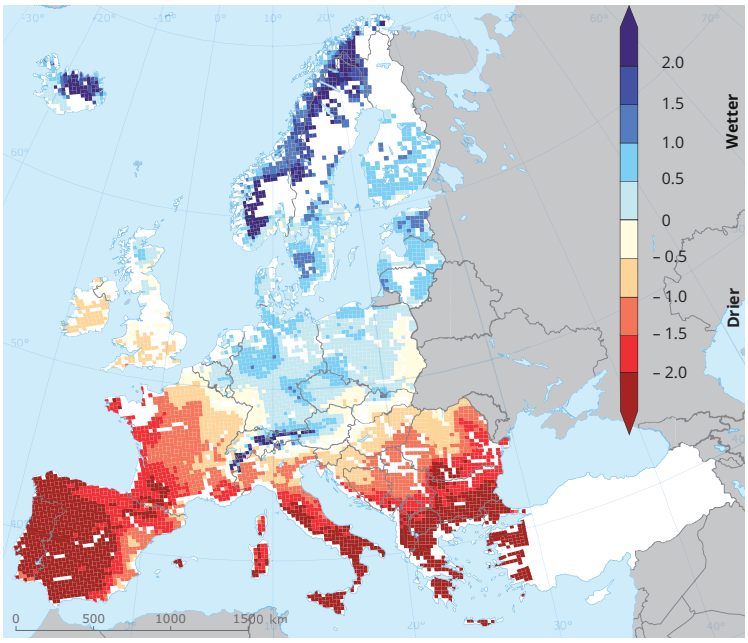

Figure 1.3: Changes in soil moisture predicted for Europe. Changes in the soil moisture are presented as mean multi-model change between 1961–1990 and 2021–2050 using 12 Regional Climate Models. Red colours indicate drier and blue colours indicate wetter conditions (adopted from European Environment Agency, 2015).

Soil deliver important services for ecosystems. However, the sustainability of soil ecosystem during a drought period largely remains unclear (Geng *et al.*, 2014). The effect of drought on terrestrial ecosystems may last longer than for years, even after the termination of the drought event (Tardieu *et al.*, 2011). Although it is not possible to control the repeated occurrence of drought events, the resulting impacts can be mitigated to a certain degree.

It is very challenging to have a clear understanding of the impact of drought on the onset and end period for an ecosystem (Stahl *et al.*, 2016), mainly due to its variability and diverse array of cases. For instance, a meta analysis of natural grassland ecosystems for aboveground biomass productivity (Rustad *et al.*, 2001) revealed 19% reduction, whereas in a study on a model ecosystem (Beierkuhnlein *et al.*, 2011) no alteration was detected. Also the results for belowground biomass studies have produced inconsistent results, such as increase in root biomass being considered as a common phenomenon under drought (Kahmen *et al.*, 2005), whereas contrasting results were observed by Jentsch *et al.* (2011). Despite a large number of robust literature on drought studies of plants and soil, a comprehensive understanding of the response of terrestrial ecosystems towards drought still remains ambiguous. For example, it is not clear, which type of ecosystem will be affected the most by drought, and if the ecosystem will recover after a severe drought. Hence, in order to maintain terrestrial ecosystems to mitigate future summer drought at least to a certain degree, there is a need to understand the strategies of these ecosystems to cope with repeated occurrence of drought periods and the recovery of these ecosystems after drought.

1.2.2 Temperate grassland ecosystems

The temperate grassland ecosystem is mostly dominated by plants which rely on seasonal precipitation for their growth and survival (Sala *et al.*, 2013). The vegetation of temperate grassland ecosystems is mainly dominated by annual grassland and perennial heathland (woody dwarf shrubs, usually develop over nutrient-poor sandy soil). If there is a repeated occurrence of drought, temperate grassland and heathland ecosystems can be strongly affected (Peñuelas *et al.*, 2004; Knapp *et al.*, 2015). In the last decade, the effect of drought on temperate grassland ecosystem functions has been studied based on different plant species at different community levels. However, most of the investigations focussed either on aboveground or on belowground biomass. At the interface between plant and soil, roots play a very important role in order to supply water and nutrient to plants and incorporate plant-derived organic matter into the soil (Jones *et al.*, 2009).

The acquisition of water depends on the root system of the respective plant. The root system of the temperate grassland vegetation consists of tap and fibrous root with fine hair like structure. Jackson *et al.* (1996) suggested that approximately 83% of grassland roots biomass are found on the upper (30 cm) soil layer, where they are interconnected to the neighbouring roots and thus hold the moisture (Belter & Cahill, 2015) for the survival of plants under drought. Roots of some grassland plants can also penetrate the deeper soil layer to retain the moisture during a seasonal drought and partially release it in upper soil layers, which then can be used by other plants with

shallow root system (Kautz *et al.*, 2013).

Temperate grassland soils constitute relatively large organic C stocks and store globally around 28–37% of the terrestrial soil organic C pool (Lal, 2004). This large C pool is thought to be affected by drought (Ciais *et al.*, 2011). The relationship between above- and belowground biomass C uptake and assimilation within plants and C translocation into the soil are controversially discussed in a number of drought stress experiments leading to no general conclusions, to which degree drought influences C cycling in temperate plant-soil system. Due to the ecological and economical importance of temperate grassland ecosystem, it is crucial to improve the understanding on C cycling in the plant-soil system of the temperate grassland ecosystems (Chambers *et al.*, 2016).

1.2.3 The carbon cycle in the plant-soil system

Atmospheric carbon dioxide (CO_2) is taken up by plants through photosynthesis (Figure 1.4). Plants are the main source for C input in soil. In the past, leaf biomass was assumed to be the main source of soil organic matter. However, roots translocate variety of plant-derived organic matter (Jones & Donnelly, 2004) from shoots towards the soil. Transfer of C from plants to soil is strongly depends on vegetation, the pattern of C allocation and assimilation within the plant and further degradation of organic matter into the soil by microorganisms (Jobbágy & Jackson, 2001). This is collectively influenced by physical, chemical and biological processes of C cycling in the plant-soil system (Schmidt *et al.*, 2011).

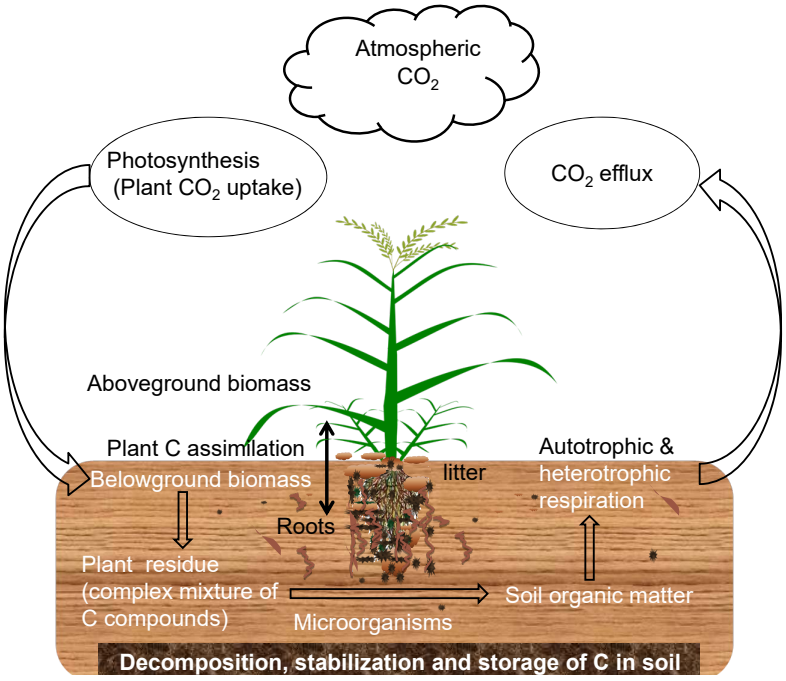

Figure 1.4: Simplified scheme of C cycle in the plant–soil system. The atmospheric CO_2 is used by plants for photosynthesis. The photosynthetically fixed C is allocated within the above- and belowground biomass, where C is used for the growth and maintenance of the plant biomass. Plant biomass is the main source of the transfer of organic matter in soil. C is released back to the atmosphere by autotrophic (shoots, roots) and heterotrophic respiration (soil microorganisms).

Drought has a major impact on the processes responsible for C cycling in the plant-soil system. A great interest has arisen to understand drought effect on soil C storage. This has emerged as a very important topic, because storing C into the soil can improve soil health and mitigate climate change (Lal, 2016). Soil store three times more C compared to vegetation (Ontl & Schulte, 2012). However, drought conditions may result in a shift in the magnitude and pattern of C cycling in the soil (Tardieu *et al.*, 2011). But no clear pattern was observed if drought promotes the soil C storage or not. Apart from C, drought also influences N availability in soil, which is an important nutrient and necessary for plant growth. Since photosynthetic activity is associated with leaf N concentration, a balance of N in the plant-soil system is important (Larsen *et al.*, 2011). Some studies reported that N availability in soil was reduced during an extended drought period (Borken & Matzner, 2009; Larsen *et al.*, 2011). Hence, there is a strong requirement to improve the understanding on the C and N cycling in the plant-soil system. Therefore, the main goal of this thesis was to understand the effect of different duration of a summer drought on the pattern of the short-term C dynamics in the plant-soil system.

1.2.4 Plant responses to drought and role of lipids

Plant tolerance to drought and responses at the biochemical and genetic levels have been frequently studied (Tardieu *et al.*, 2011). Despite a strong improvement could be achieved in the understanding of complex physical, chemical and molecular response of plants towards initial to moderate drought (Harb *et al.*, 2010), it still remains questionable how long plants can survive under drought and how plant-derived C storage in soil will change under drought.

Plants have several strategies to cope against drought which normally involves 'drought avoidance' (by lowering stomatal conductance and investing high root/shoot ratio) and 'drought tolerance' strategy (through accumulation of carbohydrate reserve in plant tissues (Kooyers, 2015)), which depends on the plant C allocation pattern (Kleczewski *et al.*, 2010). For example in water and nutrient limited soil, higher C allocation is expected towards roots for the acquisition of nutrients (Kleczewski *et al.*, 2010) and thus high C allocation towards soil can be observed (Sanaullah *et al.*, 2012). This indicates that drought periods can have the potential to increase the belowground C allocation.

In general, several studies have been conducted to understand the drought response of different plant-derived organic compounds in the aboveground biomass that have been shown to contribute in the storage of C in soil, but very few studies have reported the impact of drought in the belowground biomass. At the interface between plant and soil, the response of above- and belowground biomass depends on several factors. For instance, under drought conditions, plant invest more C for root growth compared to that of shoot growth and the maintenance of the shoots (Kleczewski *et al.*, 2010). Moreover, the C is used for the formation of more secondary compounds, which are required for energy storage and protection of plants (Zingaretti *et al.*, 2013). Among all secondary compounds, lipids are regarded as the main component of the cell membranes and cuticular waxes in the plants (Ohlrogge & Browse, 1995). The outermost surface of the plant covered with a cuticular wax layer, which is hydrophobic in nature. Epicuticular

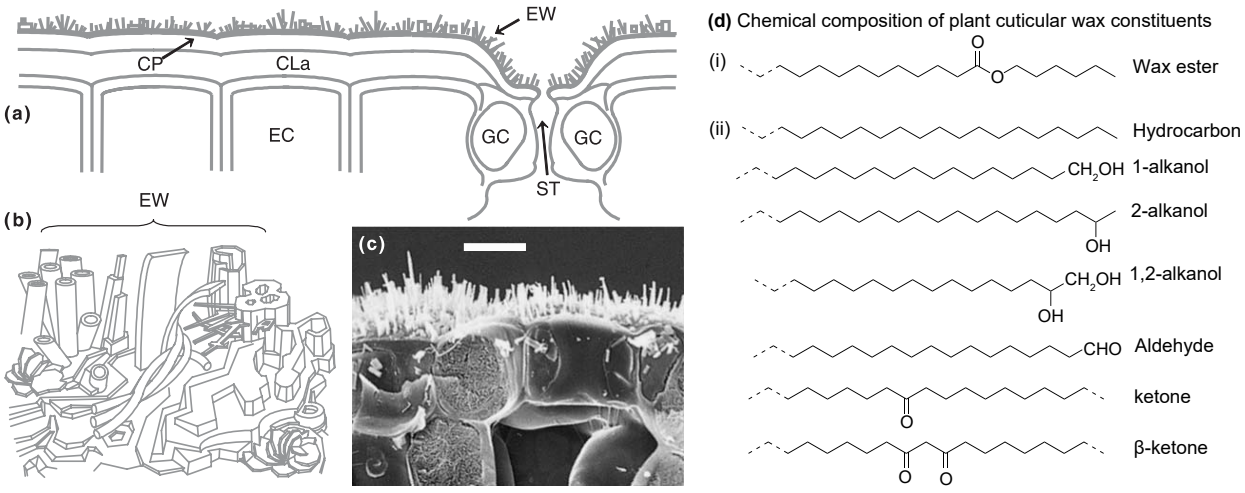

Figure 1.5: Structure and chemical composition of leaf cuticular waxes. (a) Plant leaf cross-section showing epicuticular wax (EW); cuticle proper (CP) consisting of epicuticular wax, intracuticular wax and cutin; cuticular layer (CLa) consisting of intracuticular waxes, cutin and polysaccharides; epidermal cells (EC); guard cells (GC) and stomata (ST). (b) Different crystal forms observed for epicuticular wax. (c) Scanning electron micrographs showing leaf freeze-fracture cross-sections of Kale, *Brassica oleracea*, cv. Fribor. Adopted from Shepherd & Griffiths (2006). (d) Chemical composition of plant cuticular wax constituents.

wax layer consists of mainly straight-chain aliphatic hydrocarbons with a variety of substituted functional groups (Figure 1.5) that protect plants against drought stress (Shepherd & Griffiths, 2006). The cell membranes are the main target of the degradative process under drought (Jenks & Hasegawa, 2005), while production of more cuticular waxes can be expected.

Increased amount of epicuticular wax is associated with enhanced drought tolerance and reduced water loss from the plants (Yang *et al.*, 2011). Epicuticular wax represents a complex mixture of (normal) *n*-alkanes, *n*-aldehydes, fatty acids and *n*-alcohols and several other chemical constituents (Eglinton *et al.*, 1962; Lichtfouse *et al.*, 1998) as shown in (Figure 1.5). Among all lipid components found in plants, *n*-alkanes comprises a straight hydrocarbon with homologous series of long-chain C compounds (ranging from C_{20} to C_{40}). Long-chain *n*-alkanes ranging from more than 25 C atoms mainly derived from epicuticular waxes of higher plant biomass (Eglinton *et al.*, 1962; Kolattukudy *et al.*, 1976). In contrast to these compound short- and mid-chain homologues with less than 25 C atoms in the chain length are derived from multiple sources including roots (together with microorganisms) of higher plants or microbial biomass (Kolattukudy *et al.*, 1976; Lichtfouse *et al.*, 1998).

Among all lipid compounds, *n*-alkanes are the most easily detectable components and frequently used as biomarkers (Lichtfouse *et al.*, 1998). Furthermore, *n*-alkanes can be used for source determination of plant- and microorganism-derived organic matter and can get preserved in the soil (Amelung *et al.*, 2008). They can be regarded as a part of the intermediate stable C pool in soil (Cayet & Lichtfouse, 2001). Subsequently, these *n*-alkane biomarkers have been frequently applied in fundamental ecological research studies (Salasoo, 1987; Gigon *et al.*, 2004; Wiesenberg *et al.*, 2004; Wang *et al.*, 2007).

Table 1.1: Literature survey on plant lipid response during drought. The experiments dealing with water withdrawal for a defined period of time.

Plant species	Drought manipulation	Field / laboratory	Lipid composition	References
Gossypium hirsutum	Mild, severe	Laboratory	–	Anh *et al.*, 1985
Brassica napus	10 days	Laboratory	–	De Paula *et al.*, 1990
Carthamus tinctorius	6–10 days	Laboratory	+/–	Hamrouni *et al.*, 2001
Ramonda serbica	21 days	Field	–	Quartacci *et al.*, 2002
Arabidopsis thaliana	14 days	Laboratory	–	Gigon *et al.*, 2004
Medicago truncatula	21 days, 3 cycle	Green house	+	Zhang *et al.*, 2005
Nicotiana glauca	9 days, 3 cycle	Laboratory	+	Cameron *et al.*, 2006
Sesamum indicum	14 days	Field	+	Kim *et al.*, 2007
Carum carvi	Moderate, severe	Field	–	Laribi *et al.*, 2009
Arabidopsis thaliana	14 days	Laboratory	+	Kosma *et al.*, 2009
Oryza sativa	3 days	Laboratory	+	Islam *et al.*, 2009
Arabidopsis thaliana	20 days	Laboratory	+	Seo *et al.*, 2011
Jatropha mollissima	1 year, 1 cycle	Field	+	Figueiredo *et al.*, 2015
Triticum aestivum	2 years, 1 cycle	Field	+	Guo *et al.*, 2016

Theoretically, increased lipid production in plants and its subsequent incorporation in soil can be expected under drought. Plant lipid composition is widely studied in drought experiments (Table 1.1). However, the response of lipid biosynthesis and plant lipid composition under drought is not uniform for all aboveground biomass (Table 1.1) and no such data is available for root lipids. An increased production of *n*-alkanes was determined in plants (for example *Arabidopsis thaliana*) under drought (Kosma *et al.*, 2009; Seo *et al.*, 2011). But the results were inconsistent when compared with other studies (Gigon *et al.*, 2004). Moreover, such investigations have not been described for lipids in root biomass. Recently, root-derived lipids and *n*-alkanes in soil have been considered as a significant source of plant lipids (Mendez-Millan *et al.*, 2010; Wiesenberg & Gocke, 2017). The importance of root-derived lipids in soil was largely underestimated due to earlier assumption that soil lipids derive mainly from abraded plant waxes and litter fall and its degradation (Cranwell, 1981; Zech *et al.*, 2009). The degradation and stabilization mechanism of *n*-alkanes are expected to be changed due to change in climate (Feng *et al.*, 2008).

The different sources (shoots, roots or microorganisms) and degree of degradation of *n*-alkanes in soil can be estimated using several parameters such as the average chain length (ACL) and the carbon preference index (CPI) (Wiesenberg & Gocke, 2017). Additionally, compound-specific isotope analysis on *n*-alkanes are widely used to determine the time and production in plants and its storage in soil (Collister *et al.*, 1994; Wiesenberg *et al.*, 2004; Amelung *et al.*, 2008). For soil research, main focus is given on the significance of plant-derived *n*-alkanes due to their characteristic chemical properties and comparatively slower turnover in soil (Cayet & Lichtfouse,

2001; Wiesenberg *et al.*, 2004; Flessa *et al.*, 2008; Marschner *et al.*, 2008). Despite the availability of numerous studies, the regulation of C uptake and its cycling in the plant-soil system is still not completely understood for bulk C and at a molecular level, especially with respect to severe drought. Hence, the following questions need to be answered:

(a) What is the limit to which plants produce lipids while dealing with drought?

(b) How does the incorporation of plant-derived lipid change in soil under drought?

1.3 Aims

To answer the above questions, my thesis attempts to contribute to the qualitative and quantitative description of modification/change in C, N and lipid composition (*n*-alkane) in temperate grassland and heathland ecosystems during the different drought phases. To the best of my knowledge, till date, no drought studies have been investigated beyond a continuous time-frame of 50–60 days of the experimental drought period under field conditions. Hence, this thesis presents a study on the drought-induced potential impacts on C uptake, assimilation and translocation during 104 days of a summer drought (a duration unparalleled in European history). In addition, this thesis provides new findings in the area of fundamental research to understand the recovery of the C cycle in the plant-soil system under predicted frequent cycle of extreme drought and rainfall events.

The research project was based on the EVENT I experiment on model (artificial) grassland and heathland ecosystems. A methodological approach included three times $^{13}CO_2$ pulse-chase labelling during three different drought phases to trace the plant C uptake and its incorporation into the soil. The overall main aim of this thesis was to examine the drought-induced response on C and lipid dynamics at a molecular level and the recovery of model grassland and heathland ecosystems exposed to different duration of the drought followed by irrigation. This main objective of the thesis is divided into three major research questions (corresponding to three Manuscripts), each guided by two hypotheses which are as follows:

Question 1: What is the effect of 100–1000 year extreme repeated annual drought on the C and lipid compositions in the plant-soil system? (Manuscript I)

Hypotheses

(i) Annual grassland plants in the investigated ecosystems do not sustain a change in their lipid composition when exposed to annual drought, whereas perennial heathland plants do.

(ii) One year after the five years of repeated annual drought, the soil C concentration would be expected to decrease, whereas lipid concentration increases relative to the C concentration.

Question 2: How does a severe drought affect lipid composition (*n*-alkanes) in the plant-soil system in a grassland and heathland ecosystem? (Manuscript II)

Hypotheses

(i) Increase in lipid concentration, *n*-alkane chain length as well as higher $\delta^{13}C$ values in shoots, roots and a minor increase in soils are expected under drought.

(ii) Stronger changes are expected for temperate grassland than for heathland ecosystem when they are exposed to drought.

Question 3: What is the impact of a severe drought on the short-term C dynamics in the plant-soil system in grassland and heathland ecosystems and how do these ecosystems respond to irrigation after drought? (Manuscript III)

Hypotheses

(i) Plant C uptake and transfer towards soil decreases with increase in the duration of the experimental drought.

(ii) Irrigation after long-term drought in the plant-soil system can re-activate the C cycling in the plant-soil system.

2 Methodology

To answer the research questions, model grassland and heathland ecosystems were chosen (Figure 2.1). In the context of the current study, a model ecosystem is an artificially made ecosystem with limited number of species and provides an opportunity to understand the response of plants under different phases of a severe drought (Sala *et al.*, 2013). Plants and soil samples were collected from a field experiment (EVENT I), where model grassland and heathland ecosystems were exposed to 100–1000 year extreme repeated annual drought from 2005–2010 during the peak growing season (April-September) of plants (Jentsch *et al.*, 2007).

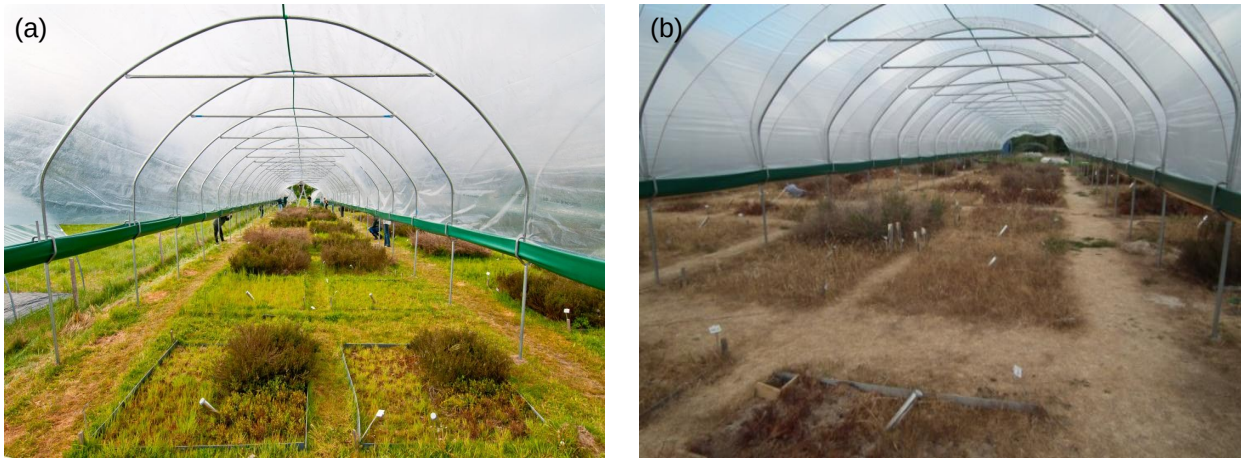

Figure 2.1: Model grassland and heathland ecosystems in the EVENT I experiment,where a severe drought was simulated. (a) Green and healthy grassland and heathland plants at the beginning of the severe drought experiment in May 2011. (b) All plants in the grassland and heathland ecosystems turned brown after severe drought treatment for 104 days (August 2011).

The repeated annual drought simulation in the EVENT I experiment provided a great opportunity to study the recovery of the plant-soil system after a drought period in terms of their C, N and lipid dynamics (Manuscript I). The plants and soil samples were collected on 18^{th} May 2011, i.e. one year after five years of the repeated annual drought. Furthermore, as an extension of a drought study in the model ecosystems, a severe drought experiment (May-September 2011) was conducted to improve the understanding of the drought-induced changes in C dynamics and utilization of C at a molecular level (Manuscript II). During the different phases of a severe drought (Figure 2.3), freshly assimilated C allocation and short-term C dynamics were studied, using a conceptual approach of $^{13}CO_2$ pulse-chase labelling (Figure 2.5). Furthermore, an irrigation experiment followed after the severe drought provided a unique opportunity to determine the time required for the re-activation of the C cycling in the plant-soil system that was accumulated during the applied drought period (Manuscript III). To achieve goals of Manuscript II and III, plant and soil samples were sampled weekly (for aboveground biomass) and biweekly (for belowground biomass and soil) during the severe drought phases and continued until the end of September 2011 (supplementary data Manuscript III).

2.1 Field experiment

The experimental site was located in Bayreuth, Germany (Jentsch *et al.*, 2007, 2011). Table 2.1 provides an overview of the main features of the EVENT I experiment. The different extremes and duration of the applied drought period is summarized in Table 2.2. Since other experiments and instrumentation were conducted on the same study site, only plant communities consisting of several plant species were available for the current study. A model grassland community consisted of four grassland plants (*Planatgo lanceolata*, *Holcus lanatus*, *Lotus corniculatus* and *Arrehenatherum elatius*) and a model heathland community consisted of two plants (*Calluna vulgaris* and *Vaccinium myrtillus*) as shown in Figure 2.2. *Arrehenatherum elatius* was not included in the study. Data acquisition was carried out in the central square meter of each plot only, in order to circumvent edge effects. In this field experiment, five consecutive years of repeated annual drought was followed by severe drought and irrigation treatment to study 32 different response parameters, that are strongly influenced by drought (Jentsch *et al.*, 2011) such as primary production, nutrient cycling, C fixation, water regulation and community stability (Figure S.1).

Table 2.1: A brief overview of the EVENT I experiment. Data compiled from Jentsch *et al.* (2007, 2011)

Characteristics	Information
Location	49°55.19′ N 11°34.55′ E, 365 m a.s.l. (Bayreuth, Germany)
Mean annual temperature	8.2°C
Mean Annual precipitation	724 mm
Experimental type	Field experiment
Experimental design	Latin square
Treatment applied	Drought and control[a]
Field replicates	5
Plots studied	20[b]
Plot size	$2m \times 2m$
Ecosystem type	Model ecosystem
Vegetation type	Grassland, heathand
Grassland plants	*Plantago lanceolata, Holcus lanatus, Lotus corniculatus, Arrhenatherum elatius*[c]
Heathland plants	*Vaccinium myrtillus, Calluna vulgaris*
Soil type	Produced from homogenised substrate from a sand quarry
Soil texture/mass	Sandy loam
Sand	82%
Silt	13%
Clay	5%
Upper soil layer (0–20 cm) pH (1 M KCl)	4.5
Lower soil layer (20–80 cm) pH (1 M KCl)	6

[a]Other treatments applied in EVENT-I experiment have not been included in this study.
[b]Out of 60 drought and control treated plots from EVENT-I experiment, only 20 plots were used for this study.
[c]Not included in this study due to quick loss of this plant species.

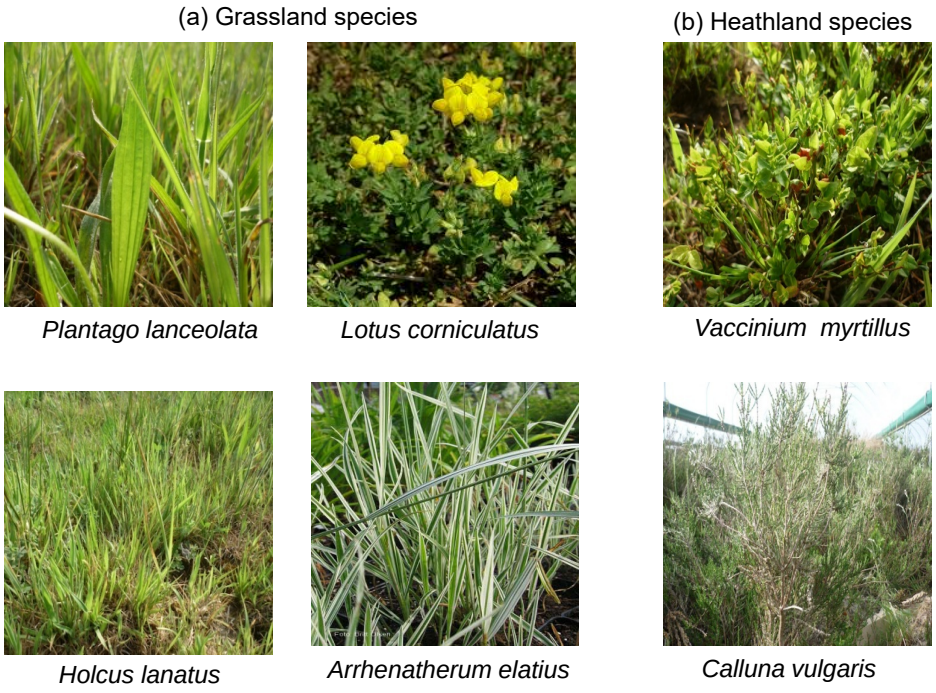

Figure 2.2: Grassland and heathland plant species used for the study. For grassland, *Plantago lanceolata*, *Lotus corniculatus*, *Holacus lanatus* were used. *Arrhenatherum elatius* was not included in the study due to its quick disappearance from the experimental plots after 30 days of the drought. For heathland, *Vaccinium myrtillus* and *Calluna vulgaris* were used.

2.1.1 Drought manipulation

For the drought simulation in EVENT I experiment, drought was defined as a number of consecutive days with <1 mm daily precipitation and simulated as complete rainfall exclusion underneath rain-out shelters. As shown in Table 2.2, five years of repeated annual drought were applied. The intensities of the repeated annual drought treatment (32 days/year from 2005–2007) was based on the local 100 and (42 days/year from 2008–2010) 1000 year extreme. Alteration in precipitation and volumetric soil water content was weakly measured (Jentsch *et al.*, 2011).

Table 2.2: Experimental drought and irrigation treatments in the EVENT I experiment. Modified after Backhaus *et al.* (2014)

Type of treatment	Date of treatment	Year	Extreme	Days
Repeated annual drought	June 6 – July 10	2005	100 year	32
	May 25 – June 24	2006	100 year	32
	May 20 – June 20	2007	100 year	30
	May 19 – June 30	2008	1000 year	42
	May 19 – June 29	2009	1000 year	42
	May 11 – June 21	2010	1000 year	42
Severe drought treatment	May 17 – August 28	2011	Unprecedented	104
Irrigation treatment	August 29 – September 20	2011	Regular watering	22

The control plots remained without weather manipulation throughout the entire investigation period (years 2005–2010) and were maintained by regular irrigation using a portable irrigation system (Kreyling *et al.*, 2008b; Jentsch *et al.*, 2011) throughout the drought manipulation period if the precipitation was below the common annual precipitation to avoid drought periods on these plots. These plots received only natural precipitation. Therefore, soil moisture concentration strongly differed during drought manipulations (Backhaus *et al.*, 2014).

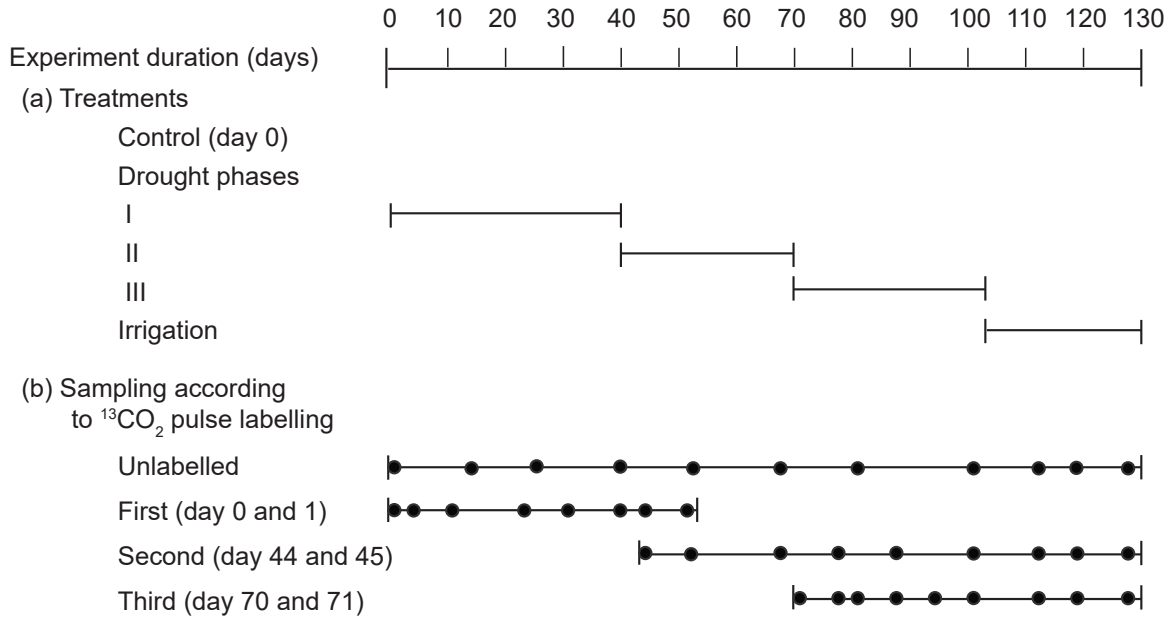

Figure 2.3: Different phases of the drought and irrigation. Experimental scheme showing (a) three different phases (I, II and III) of drought followed by irrigation and (b) sampling performed on unlabelled and labelled (after each $^{13}CO_2$ pulse labelling) plots. Dots represent the day of sampling (for detail information about sampling see supplementary data Manuscript III).

On 11–13th May 2011, all plots received a watering treatment to adjust the same initial condition (Backhaus *et al.*, 2014). Afterwards, a very severe drought (exceeding projected climate change scenario) was applied to all plots that experienced different pre-treatments like annual drought or control conditions Figure 2.1a and b. Since all plots were covered by one large rain-out shelter, there were no control plots available in parallel to the drought investigation period. I am aware of the limitation of this approach. The performance of the model grassland and heathland ecosystems at day '0' was considered to show the maximum potential level of the plant-soil system. It was assumed that this maximum potential level would remain stable throughout the drought investigation period under control conditions. The simulated severe drought was divided into three different phases (Figure 2.3a and b) imposed on the model ecosystems from initial to moderate and followed by a strong drought period within one growing season (May-September 2011). Days 0–40 was termed as drought phase I, days 40–70 was drought phase II and days 70–104 was regarded as drought phase III and followed by an irrigation period. Three times $^{13}CO_2$ pulse-chase labelling experiments were conducted during three different drought phases. Subsequently, plant and soil samples were collected throughout the complete drought period. Furthermore, sampling continued even after the irrigation, which was applied on 29th August 2011 to all plots.

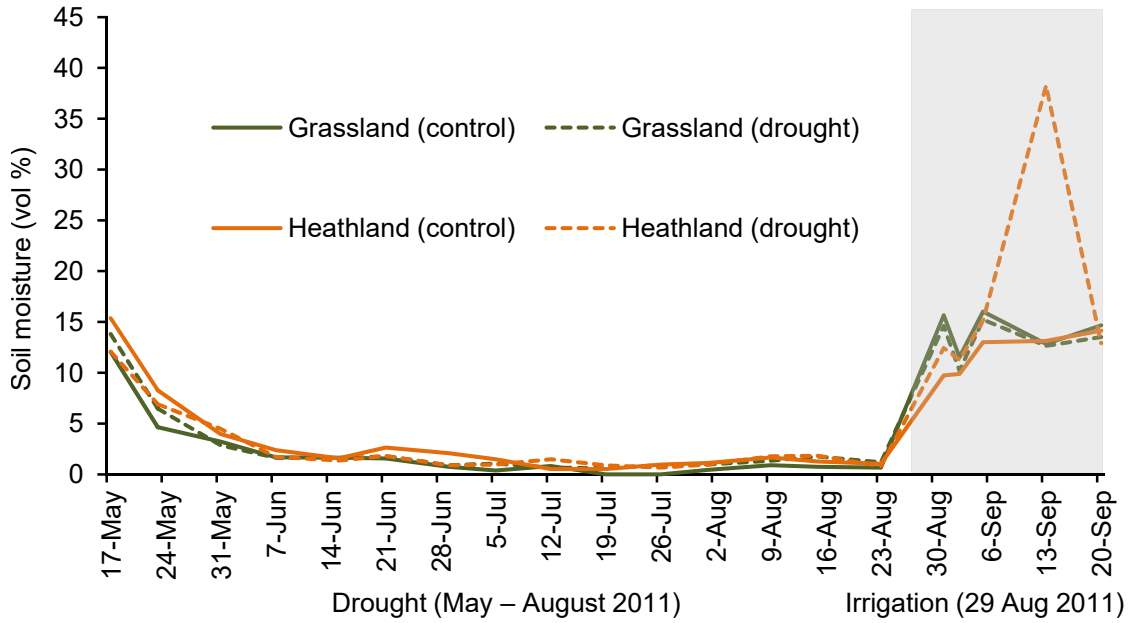

Figure 2.4: Changes in soil moisture under drought and irrigation. Volumetric soil water concentration (vol%). The weekly mean was observed over the course of the very severe drought and irrigation (gray box) experiment in the year 2011. Modified after Backhaus *et al.* (2014).

During the severe drought period soil moisture was the most limiting factor for plant-growth. It was measured weekly on each plot at a depth of 10 cm (Jentsch *et al.*, 2011; Backhaus *et al.*, 2014). The volumetric soil water content of all plots dropped below the permanent wilting point (7 Vol%) on 26^{th} May 2011 and remained below the permanent wilting point for the rest of the drought period. The soil water content for all plots returned back to its normal level (soil volumetric water content >10%) after irrigation (Figure 2.4).

2.1.2 Pulse-chase labelling experiment

Among several other applications of stable isotopes in ecological research, one very frequent application of stable isotopes is to use them as tracers. The isotopic ratio of an element is expressed as abundance ratio of heavier to lighter isotope (R), which is the ratio of $^{13}C/^{12}C$. The isotopic composition of a substance/sample is given relative to an international standard and expressed in per mil (‰).

$$\delta^{13}\mathrm{C}\,(‰) = \left(\frac{\mathrm{R}_{sample}}{\mathrm{R}_{standard}} - 1\right) \times 10^3 \tag{2.1}$$

where, R_{sample} stands for $^{13}C/^{12}C$ isotopic ratio of the sample and $\mathrm{R}_{standard}$ stands for the Vienna–Pee Dee Belemnite (V–PDB) (0.0111802) respectively (Dawson *et al.*, 2002).

δ^{13}C (‰) values can be determined for plant and soil and also for specific compounds i.e. lipids molecular biomarker (Wiesenberg *et al.*, 2004). Hence, the measurements of stable isotopic signature of C compound is a very powerful tool to follow the fate of C compounds among different environmental C stocks, provided that the stocks have a different C isotopic composition.

Figure 2.5: Pulse-chase labelling experiment under drought. The labelling was conducted on a clear sunny day (ca. 25 °C at noon) to ensure active uptake of CO_2 by plants. (a) $^{13}CO_2$ pulse-chase labelling on grassland plots and (b) Labelling of the plant-soil system on heathland plots.

For this thesis, a ^{13}C tracer was incorporated into the aboveground biomass via artificially created $^{13}CO_2$ atmosphere for photosynthesis and traced through the roots and soil in a model grassland and heathland ecosystem. To address the research question 3, (refer to subsection 1.3) a $^{13}CO_2$ pulse-chase labelling experiment was carried out three times during the different phases of drought in 2011. Six grassland and six heathland plots (Figure 2.5a and b) were labelled with ^{13}C by supplying the $^{13}CO_2$ atmosphere for 4–5 hours using a labelling chamber of the respective height of the vegetation height on the corresponding plots (the method description in detail is available in Manuscript II). Freshly supplied C uptake was traced in the plant (weekly) and in the soil (biweekly) after each pulse labelling experiment. C dynamics were studied in detail by calculating ^{13}C excess in shoots, roots and soil.

To express the amount of ^{13}C added by pulse labelling, atom percentages in the above- and belowground biomass and soil the following equation was used (Ruehr *et al.*, 2009)

$$^{13}\text{C atom\%} = \frac{100 \times 0.0111802 \times \left(\frac{\delta_{(\text{sample})}}{1000} + 1\right)}{1 \times 0.0111802 \times \left(\frac{\delta}{1000} + 1\right)} \tag{2.2}$$

where, $\delta_{(\text{sample})}$ represents the isotopic values of labelled or unlabelled shoot, root and soil samples. For all shoot, root and soil samples, the excess ^{13}C was calculated for grassland and heathland ecosystems, taking into account the dry biomass pools in plant and soil compartments.

$$\text{Excess } ^{13}\text{C}_{(\text{sample})} = \frac{\text{atom\%}_{(\text{labelled})} - \text{atom\%}_{(\text{unlabelled})}}{100} \times \text{dry weight}_{(\text{sample})} \times \frac{\text{C\%}_{(\text{sample})}}{100} \tag{2.3}$$

The values were expressed as g/m^2 recovered by the $^{13}CO_2$ pulse-chase labelling in the dry weight of the plant and soil compartments and the respective C pools of the same sample compartment per ground area (each plot had an area of 1 m^2). C concentration (%) of the respective sample.

2.2 Analytical methods

An overview of the analytical methods applied in this thesis is summarized in Table 2.3. These methods are briefly described in the following chapters and further detailed information is given in the material and methods section of the respective manuscripts.

Table 2.3: Analytical methods used in the study

Analysis	Methods/equipments	References
C and N concentrations (plant and soil)	Elemental analyser (EA)[a]	Glaser, 2005
Stable $\delta^{13}C$, $\delta^{15}N$ compositions (plant and soil)	EA coupled to an isotope ratio mass spectrometer (EA-IRMS)[a]	Glaser, 2005
Total lipid extract concentration and solid phase separation of toal lipids (plants and soil)	Soxhlet extraction and SPE-clean up[b]	Wiesenberg & Gocke, 2017
Quantification of *n*-alkanes (plant and soil)	Gas chromatography-flame ionization detector (GC–FID) and GC–mass spectrometer (GC–MS)[b]	Wiesenberg & Gocke, 2017
$\delta^{13}C$ of *n*-alkanes (plant and soil)	Compound specific stable isotope analysis (CSIA) GC–coupled to an isotope ratio mass spectrometer (GC–C –IRMS)[b]	Collister *et al.*, 1994, Wiesenberg *et al.*, 2004

[a]C, N and their isotopic compositions were analysed at University of Halle–Wittenberg, Germany.
[b]Lipid extraction, quantification and CSIA of *n*-alkanes were performed at University of Zurich, Switzerland.

2.2.1 Bulk elemental and isotopic analysis

Shoots, roots and soil samples were analysed for total organic C and N concentrations as well as their isotopic ^{13}C and ^{15}N composition using an elemental analyser coupled to an isotope ratio mass spectrometer (EA-IRMS). All measurements were performed using an elemental analyser (Hekatech GmbH, Euro) coupled to a ConFlow III interface (Thermo Fisher, Bremen, Germany). Combustion of samples was followed by gas chromatographic (GC) separation and transferring sample gas to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany). C and N concentrations were given in percentage (dry weight % TOC and N) and ^{13}C and ^{15}N isotopic compositions are expressed in permil (‰), relative to V–PDB standard and calculated using the Equation 2.1.

2.2.2 Lipid analysis

Total lipid extracts (TLE) were obtained from shoots, roots and soil using Soxhlet extraction. In this method, milled plant or soil samples are placed in a porous thimble, which is placed in the Soxhlet extraction chamber. The chamber is connected upside to a condenser and down to a

round bottom flask containing a mixture of dichloromethane/methanol (93:7;v/v; CH_2Cl_2/MeOH). When the extraction solvent heats up, vapor rises up and condenses back and enters in the thimble where the sample is placed. At the time when liquid level reached to the top of the thimble, the lipid extract enriched solvent returned back to the flask. This cycle is repeated for 36 hours. The detailed procedure used for the lipid extraction and its sequential separation into three fractions (neutral lipid, fatty acid and high molecular weight compound) using solid phase extraction (SPE-clean up) with KOH coated silica gel was described by Wiesenberg & Gocke (2017). Finally, the neutral fraction of the lipid was further separated based on their polarity into aliphatic, aromatic and alcohols (Wiesenberg *et al.*, 2010; Wiesenberg & Gocke, 2017). Only aliphatic hydrocarbons (straight chain C compounds) were studied in detail.

2.2.3 Gas chromatography and mass spectrometry

The identification of *n*-alkanes, assignment of different compounds (n-C_{17}–n-C_{35}) and quantification was performed using a gas chromatograph (GC) equipped with split/splitless injector and flame ionisation detector (GC-FID; Agilent 7890B). A defined amount of a deuterated *n*-alkane standard (D_{50}–n-C_{24}) was added to the individual aliphatic hydrocarbon fractions prior to GC analysis. A number of indices of *n*-alkane molecular ratios were calculated for determination of plant-derived *n*-alkane dynamics and their contribution to soil organic matter. To characterize the modification of long-chain *n*-alkanes (n-C_{25}–n-C_{35}), the ACL was calculated as weight-averaged number of C atoms using the following equation (Poynter *et al.*, 1989).

$$\mathrm{ACL} = \frac{25[C_{25}] + 27[C_{27}] + 29[C_{29}] + 31[C_{31}] + 33[C_{33}] + 35[C_{35}]}{[C_{25}] + [C_{27}] + [C_{29}] + [C_{31}] + [C_{33}] + [C_{35}]} \tag{2.4}$$

Another parameter, which is frequently used for the description of the predominance of odd-to-even C number of long-chain *n*-alkanes (C_{25}–C_{35}), the carbon preference index CPI was calculated using the following equation (Marzi *et al.*, 1993; Diefendorf *et al.*, 2015).

$$\mathrm{CPI} = \frac{1}{2} \times \left[\frac{([C_{23}]+[C_{25}]+[C_{27}]+[C_{29}]+[C_{31}]+[C_{33}])}{([C_{22}]+[C_{24}]+[C_{26}]+[C_{28}]+[C_{30}]+[C_{32}])} + \frac{([C_{23}]+[C_{25}]+[C_{27}]+[C_{29}]+[C_{31}]+[C_{33}])}{([C_{24}]+[C_{26}]+[C_{28}]+[C_{30}]+[C_{32}]+[C_{34}])} \right] \tag{2.5}$$

Thus, CPI values reflect the differences of the relative concentrations between odd to even *n*-alkanes homologous. High values (>10) are typical for fresh leaf biomass, while values close to 1 indicate strong degradation of organic matter (Cranwell, 1981).

2.2.4 Compound specific isotope analysis of *n*-alkanes

Compound-specific isotope analysis (CSIA) is a common source allocation tool which uses the incorporation of stable isotopes into biomolecules and byproducts that are generated during the biochemical processes associated with degradation (Collister *et al.*, 1994; Cayet & Lichtfouse, 2001). The modification in $\delta^{13}C$ composition of *n*-alkanes in plants and soil were studied to track changes when the ecosystems are exposed to drought. The stable $\delta^{13}C$ isotopic ratios of individual

n-alkanes were determined using a ThermoScientific Trace 1310 gas chromatograph interfaced on-line via a GC-Isolink II to a ConFlow IV-Delta Plus isotope ratio mass spectrometer. Isotopic composition of *n*-alkanes are reported as δ^{13}C values relative to V-PDB averaging at least three replicate measurements. The averaged values of the CSIA results were used for the calculation of the C proportions of mean of the 5 most abundant compounds (C_{25}, C_{27}, C_{29}, C_{31}, C_{33}) normalized to the proportion of each compounds (Wiesenberg *et al.*, 2008).

$$\text{Weighted average of } n\text{-alkanes}\,(‰) = \frac{(\mathrm{A} \times \delta_\mathrm{A}) + (\mathrm{B} \times \delta_\mathrm{B}) + (\mathrm{C} \times \delta_\mathrm{C}) + (\mathrm{D} \times \delta_\mathrm{D}) + (\mathrm{E} \times \delta_\mathrm{E})}{\sum(\mathrm{A{:}E})} \tag{2.6}$$

where A, B, C, D and E represent the relative proportion of the most abundant compounds and δ_A, δ_B, δ_C, δ_D, δ_E as their δ^{13}C isotopic values.

2.2.5 Statistical analysis

The mean and the standard error are based on five field replicates (Webster, 2001) for Manuscript I. Since one year after five years of repeated annual drought, no significant effect was observed therefore, repeated annual drought and control treated plots were used as an independent field replicates. Hence, for Manuscript II and Manuscript III the mean and standard errors are based on 10 field replicates, except for the labelling experiment, where only 6 field replicates were available.

The bulk C, N, TLE concentrations, CPI and ACL were tested for significant differences using a parametric test, one-way Analysis of Variance (ANOVA) (Webster, 2007). To test the differences between control (day 0) and drought for each individual parameter, a linear mixed effect (lme) model was used with community response and treatment as fixed effect, and replication as random effects for each sampling date. The test was performed using ANOVA and a significance level of $p < 0.05$, followed by post hoc Scheffe´ test. Furthermore, Student's t-test with a significance level of $p < 0.05$ was applied to test the difference between grassland and heathland communities. The statistical evaluation was performed with R (R Development Core Team, 2014).

3 Results and discussion

3.1 Manuscript I: Repeated annual drought had no long-term influence on C and lipid dynamics (Srivastava *et al.*, 2017a)

Under natural field conditions, it may take several months or even years for a plant to repair damaged root systems and regain its growth after a drought (Schimel *et al.*, 2007; Borken & Matzner, 2009; de Vries *et al.*, 2012). This recovery of ecosystems strongly depends on the intensity and the duration of the applied drought period (Hoover, 2014). Currently, no general information is available on the relationship between above- and belowground C cycling processes influenced by drought and its recovery at a molecular level. To answer the first research question of this thesis (question 1, subsection 1.3), the first study of this kind was performed to investigate the long-term influence of five years of repeated annual drought on sustainable changes in C, N and lipid compositions in temperate grassland and heathland model ecosystems.

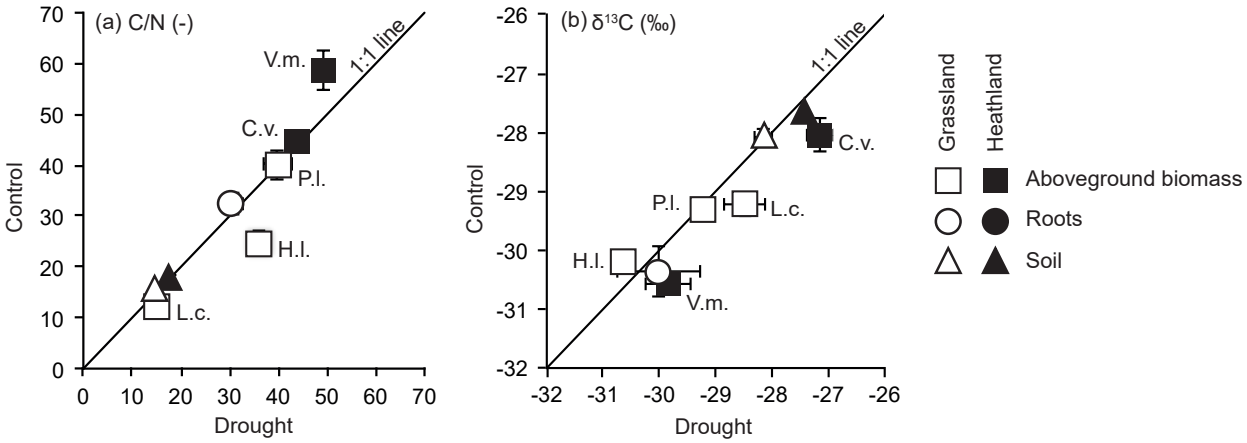

Figure 3.1: Repeated annual drought had no long-term effect on C and N dynamics. (a) C/N ratio and (b) stable Carbon (C) (δ^{13}C) isotope composition under control and one year after repeated annual drought treatment. Values (n=5) are given as the mean with the standard errors. Abbreviations: V.m. (*Vaccinium myrtillus*), C.v. (*Calluna vulgaris*), P.l. (*Plantago lanceolata*), H.l. (*Holcus lanatus*), L.c. (*Lotus corniculatus*).

It was expected to observe a change in C and N concentrations in plants and soil (Asner *et al.*, 1997) due to reduced C uptake (Chaves *et al.*, 2003) by plants and low N availability in the soil under a drought (Mikha *et al.*, 2005). But no significant change was observed in terms of C and N concentrations in plants nor in the soil one year after the recurrent annual drought (Figure 3.1a). This result was not surprising if I take into account the unchanged plant productivity and soil microbial activity observed in the same experiment (Kreyling *et al.*, 2008a,b; Jentsch *et al.*, 2011, Figure S.1 supplementary information). However, this is inconsistent with other field studies (Olesen & Bindi, 2002; Peñuelas *et al.*, 2004; Ciais *et al.*, 2005).

Furthermore, the stable C isotope composition was expected to be increased in plants that frequently experience drought events due to the adaptation of the plants after repeated droughts (Farquhar *et al.*, 1989). However, no significant change was observed in any investigated plant species except for *C. vulgaris* (Figure 3.1b). This is most probably related to the C stored in

the woody tissue of this perennial heathland plant (Gerdol *et al.*, 2000). On the other hand, since grassland plants renew their plant biomass annually, no traces of accumulated C from the previous year could be expected. No significant changes were observed in the stable C isotope composition of either the roots or the soil one year after the repeated annual drought. This suggested that 32–42 days of drought per year did not have a significant effect on the C budget in soil, although these 100 and 1000 year extremes in terms of drought duration might have strong impacts on the biodiversity (Kreyling *et al.*, 2008b).

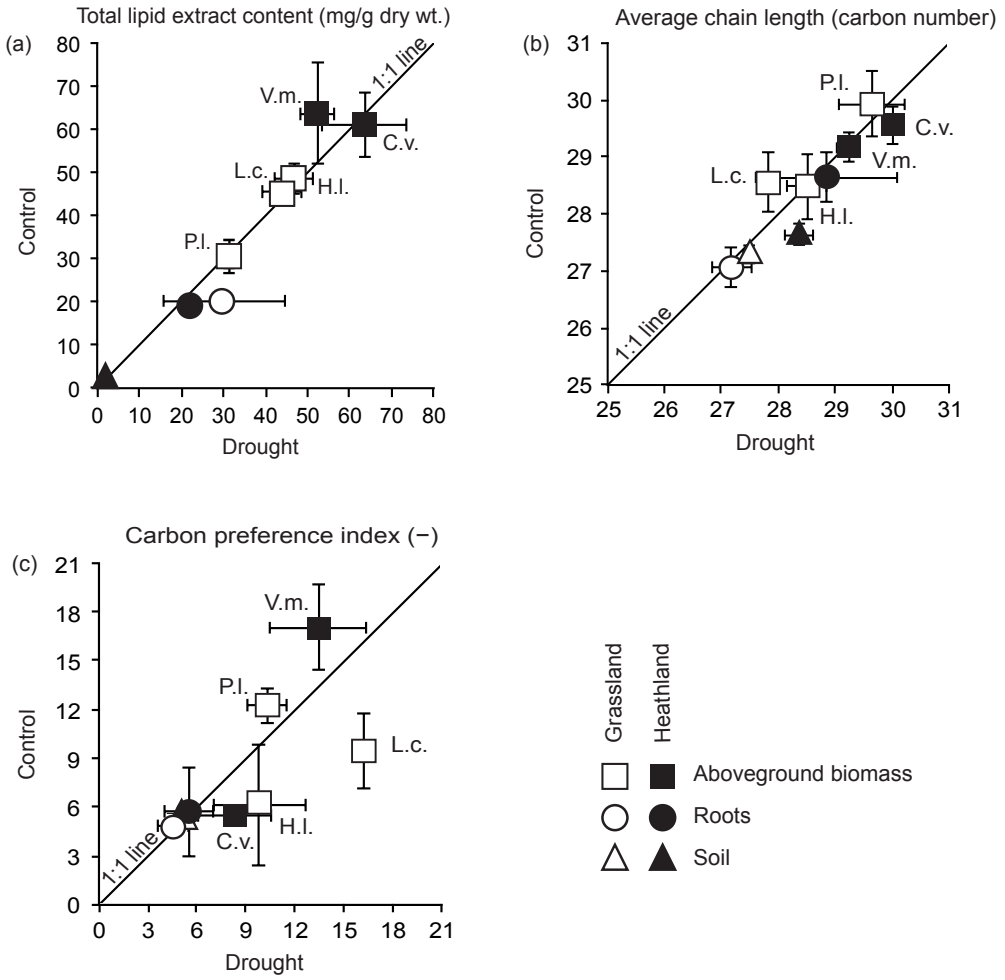

Figure 3.2: Influence of drought on TLE, CPI and ACL. (a) Total lipid extract (TLE) concentration (mg/g dry weight), (b) ACL and (c) CPI under control and one year after repeated annual drought treatment. Values (n=5) are given as the mean with the standard errors. Abbreviations: V.m. (*Vaccinium myrtillus*), C.v. (*Calluna vulgaris*), P.l. (*Plantago lanceolata*), H.l. (*Holcus lanatus*), L.c. (*Lotus corniculatus*).

At a plant-metabolic level, carbohydrate as well as amino-acid concentrations were reported to decrease under drought (Lawlor & Cornic, 2002). Thus, C available in the plant could be used to synthesize other secondary metabolites such as lipids (Zingaretti *et al.*, 2013), which protect the plants against drought (Kosma *et al.*, 2009). For soil, long-chain *n*-alkanes have the potential to be selectively preserved in the soil compared to other compounds (Lichtfouse *et al.*, 1998). Therefore, increased TLE concentration and long-chain of *n*-alkanes was expected even one year after the repeated annual drought (Shepherd & Griffiths, 2006). Although heathland shoots, roots and soil

revealed high lipid concentrations, surprisingly TLE as well as various *n*-alkane molecular ratios did not significantly change in most of the shoots under drought vs. control (Figure 3.2). An exception was observed in *L. corniculatus* which showed significant increase in CPI and decrease in ACL. This indicated a reduction in the chain length of *n*-alkanes, but probably an increased production of short-chain *n*-alkanes with more byproducts like even homologues one year after several drought seasons.

Missing changes in *n*-alkane composition in other shoots suggested a fast recovery of *n*-alkane production in plants other than *L. corniculatus*. Hence, for aboveground biomass, changes in the alkane biosynthesis occurred one year after the recurrent drought, but an increase in the chain length of *n*-alkanes was not observed contrary to previous observations (Shepherd & Griffiths, 2006). No significant change in *n*-alkane composition was observed for grassland and heathland soil. Although the available time series for the current study was too short to achieve any general conclusion for drought-related changes in soil, the observed finding suggests that even after a recurrent annual drought (100 and 1000 year extreme), a plausible and fast recovery of C cycling and resilience of plant-derived lipids in the plant-soil system can be possible.

3.2 Manuscript II: The lipid composition of plants and soil quickly responded to drought in grassland and heathland ecosystems (Srivastava & Wiesenberg, under revision)

In order to understand the role of *n*-alkanes in plants exposed to drought, it is essential to examine changes not after the drought period but during drought events. Furthermore, if the ecosystems recover fast in terms of C and lipid concentrations as observed in Manuscript I, the question remains: what time period is required for an improved biosynthesis of lipids, their assimilation within plants and their incorporation into the soil? In this context, the research question 2 of this thesis (question 2, subsection 1.3) was asked to understand the response of grassland and heathland ecosystems to a severe drought.

In general, lipids can be produced more rapidly (Shepherd & Griffiths, 2006) when plants are exposed to drought (Table 1.1). However, it strongly depends on the individual plant species (Salasoo, 1987; Maffei, 1996; Jenks & Hasegawa, 2005) and applied drought treatments. In order to investigate the drought-induced response of *H. lanatus* (grassland plant) and *C. vulgaris* (heathland plant), the TLE concentration and *n*-alkane distribution patterns were examined. Furthermore, the elemental and isotopic composition of bulk C and *n*-alkanes were investigated in the plant-soil system.

Significantly higher amount of TLE concentration was observed for *C. vulgaris* compared to *H. lanatus*. Higher amounts of TLE for *C. vulgaris* could be associated with the typical feature of a perennial plant which produces thick epicuticular waxes (Salasoo, 1987; Shepherd & Griffiths, 2006). A significant decrease (25–30%) in the TLE concentration in shoots, roots and soil occurred during the initial drought period (from day 0–10 days, Table 3.1). For shoots, the TLE concentration decreased after 10 days and increased to a maximum level at the end of the drought phase I (after 40 days) for *H. lanatus* and after 50 days for *C. vulgaris*. Furthermore,

Table 3.1: C and TLE concentrations in grassland and heathland ecosystems. C, TLE concentrations and TLE normalized to C_{org} in shoots, roots and soil in a model grassland and heathland ecosystems exposed to drought. Mean ± standard errors of the mean are given (n = 10). Data point on day '0' represents control.

Ecosystem	Sample	Drought phase	Sampling time (days)	C_{org} (mg/g) d.w.	TLE (mg/g) d.w.	TLE /C_{org} (mg/g) d.w.
Grassland	*H. lanatus*	I	0	512.7 ± 27.2	49.0 ± 1.7	98.5 ± 6.1
			12	499.3 ± 37.9	44.4 ± 4.0	88.9 ± 4.0
			27	495.3 ± 20.8	49.1 ± 4.1	101.8 ± 10.9
		II	40	469.9 ± 19.1	61.0 ± 7.0	124.7 ± 15.1
			54	493.3 ± 22.5	48.8 ± 2.4	99.9 ± 5.1
			68	428.4 ± 32.6	40.5 ± 1.7	101.3 ± 10.8
		III	82	468.4 ± 27.9	39.6 ± 2.3	89.3 ± 11.3
			96	498.6 ± 32.9	47.9 ± 5.4	96.6 ± 9.6
			103	435.6 ± 25.3	46.9 ± 5.8	115.7 ± 18.4
	Roots	I	0	383.8 ± 19.7	18.2 ± 1.4	48.7 ± 4.5
			12	392.2 ± 26.9	21.2 ± 1.1	55.3 ± 5.8
			27	401.7 ± 5.0	21.5 ± 1.3	53.3 ± 2.8
		II	40	430.8 ± 9.9	25.5 ± 3.5	59.1 ± 8.0
			54	542.9 ± 24.2	25.3 ± 2.4	48.7 ± 6.4
			68	386.7 ± 16.3	24.3 ± 2.6	62.1 ± 5.3
		III	82	420.6 ± 21.9	23.9 ± 4.3	56.9 ± 10.1
			96	403.6 ± 52.3	24.3 ± 1.9	63.5 ± 6.0
			103	346.9 ± 35.5	23.0 ± 3.3	67.2 ± 7.7
	Soil	I	0	25.8 ± 1.5	1.1 ± 0.1	40.9 ± 3.5
			12	18.7 ± 1.9	0.9 ± 0.0	48.3 ± 4.0
			27	30.9 ± 1.7	0.9 ± 0.0	28.9 ± 2.1
		II	40	34.7 ± 1.9	1.0 ± 0.1	26.9 ± 2.1
			54	34.4 ± 2.7	1.0 ± 0.0	32.4 ± 2.6
			68	31.3 ± 2.7	0.9 ± 0.0	31.8 ± 3.2
		III	82	32.1 ± 2.7	0.9 ± 0.0	29.8 ± 3.5
			96	33.3 ± 1.7	0.8 ± 0.1	21.5 ± 2.6
			103	32.3 ± 2.6	0.7 ± 0.0	23.5 ± 2.3
Heathland	*C. vulgaris*	I	0	527.8 ± 06.5	68.6 ± 3.3	130.2 ± 6.5
			12	499.7 ± 31.3	49.3 ± 10.3	102.6 ± 21.3
			27	537.9 ± 45.8	65.3 ± 11.0	120.9 ± 19.8
		II	40	493.7 ± 42.1	67.6 ± 3.8	146.3 ± 16.9
			54	540.1 ± 31.2	82.0 ± 4.0	168.5 ± 24.4
			68	523.4 ± 37.0	70.0 ± 4.9	136.9 ± 9.4
		III	82	564.5 ± 37.5	67.6 ± 5.9	123.2 ± 12.2
			96	648.4 ± 15.9	77.2 ± 7.6	118.8 ± 11.1
			103	591.3 ± 50.8	77.8 ± 4.4	142.9 ± 17.2
	Roots	I	0	315.0 ± 00.0	32.1 ± 3.4	94.3 ± 0.0
			12	414.7 ± 20.8	20.8 ± 1.6	50.1 ± 2.1
			27	460.9 ± 20.1	22.2 ± 2.8	48.8 ± 6.1
		II	40	514.3 ± 09.7	25.7 ± 1.2	50.1 ± 2.4
			54	564.8 ± 18.9	24.7 ± 1.8	43.6 ± 2.5
			68	469.0 ± 15.2	23.1 ± 1.5	49.6 ± 3.4
		III	82	334.5 ± 29.5	21.6 ± 2.2	64.4 ± 4.9
			96	485.9 ± 16.8	20.8 ± 3.0	43.2 ± 6.8
			103	464.8 ± 25.2	20.7 ± 1.6	42.1 ± 4.5
	Soils	I	0	24.3 ± 1.3	1.2 ± 0.1	49.4 ± 2.5
			12	17.4 ± 2.1	0.8 ± 0.1	45.0 ± 2.1
			27	35.1 ± 2.1	1.2 ± 0.1	34.9 ± 2.4
		II	40	36.8 ± 3.9	1.7 ± 0.1	47.6 ± 5.4
			54	36.6 ± 2.6	1.5 ± 0.1	44.3 ± 5.2
			68	35.4 ± 3.8	1.4 ± 0.1	42.7 ± 3.8
		III	82	28.8 ± 1.4	1.0 ± 0.1	34.1 ± 2.7
			96	30.8 ± 1.8	0.8 ± 0.1	26.1 ± 1.8
			103	28.7 ± 3.5	0.8 ± 0.0	26.6 ± 2.2

after the drought phase II, (70 days), the TLE concentration decreased for both plant species. Grassland and heathland roots did not show significant changes under drought. Soils revealed a similar trend as observed for grassland and heathland plants. Modification in TLE concentration during drought phase I, indicate that drought activated the lipid biosynthesis in plants which can be associated with the acclimatization to drought. This is in agreement with literature (Shepherd & Griffiths, 2006; Kim *et al.*, 2007; Figueiredo *et al.*, 2015).

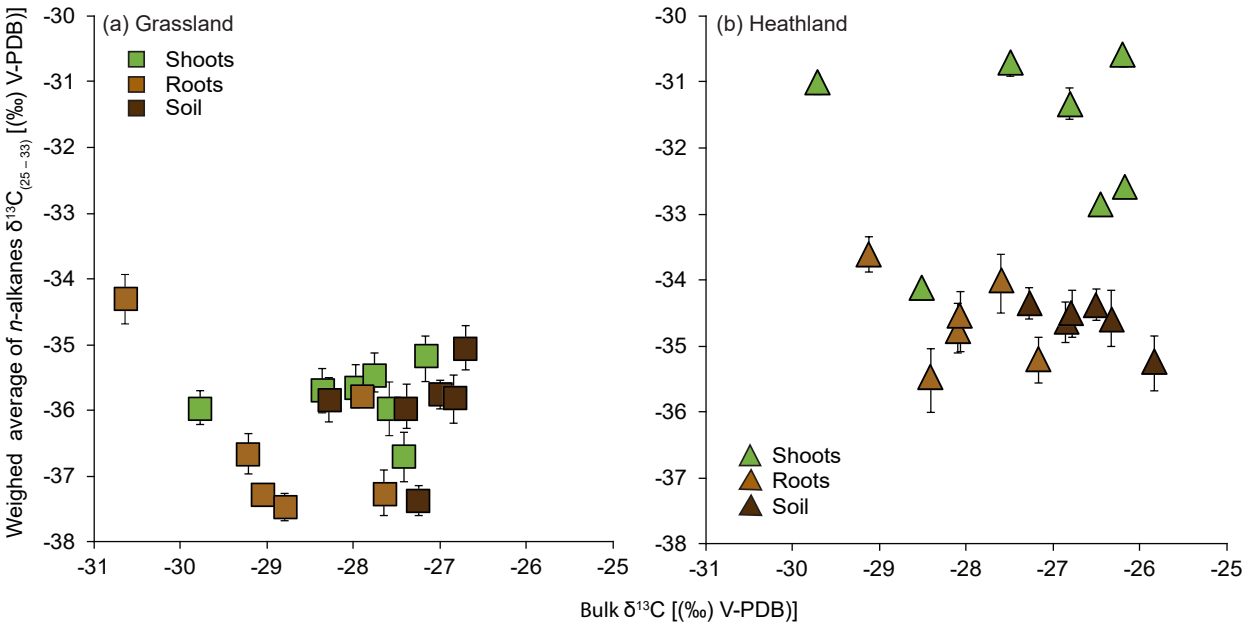

Figure 3.3: Compound-specific δ^{13}C of *n*-alkanes vs. bulk δ^{13}C in the plant-soil system exposed to drought. Weighted average isotopic composition of long-chain *n*-alkanes (C_{25}–C_{33}) compared to bulk C_{org} isotopic composition in (a) grassland and (b) heathland.

Furthermore, increased CPI and ACL of *n*-alkanes in grassland and heathland soil during drought phase I (Manuscript II) demonstrated a direct input of plant-derived long-chain *n*-alkanes into soil. This is the most surprising result of this study and had not been observed before. Since litterfall did not occur during this drought phase, increased values of CPI and ACL of *n*-alkanes in the soil indicate an improved biosynthesis of cuticular alkanes, followed by a release of excess alkanes from leaves and/or root-derived alkanes deposition in the soil.

The weighted average values of δ^{13}C of *n*-alkanes (C_{25}–C_{33}) showed 1.5‰ enrichment for *H. lanatus* (Figure 3.3) within the drought period 0–10 days. Increased values (2–3‰) for δ^{13}C as well as for CSIA of *n*-alkanes in shoots during drought phase I indicate that drought-induced stomata closures led to ^{13}C enrichment of plants. This ^{13}C was actively utilized for *n*-alkanes biosynthesis in order to withstand the drought. This can be attributed to the metabolic change in plants under drought. For *C. vulgaris*, the maximum enrichment δ^{13}C of *n*-alkanes (3‰) was observed after day 40 of the drought indicating that the *n*-alkanes were formed with the C stored previously within its woody tissues. Roots of both communities showed lower values of weighted average of δ^{13}C of *n*-alkanes under drought compared to day 0, indicating that no such formation of *n*-alkanes took place. Constant values for weighted average of δ^{13}C of *n*-alkanes (C_{25}–C_{33}) in the soil of both ecosystems indicated that potentially experiment-derived alkanes did not change

the isotope values of soil alkanes.

The current study is the first one of its kind to investigate plant metabolic responses under different phases of a severe drought at a cost of C investment for the synthesis of *n*-alkanes and rapid cycling of *n*-alkanes in the plant-soil system. The increase in lipid concentration and chain length of *n*-alkanes as well as higher compound-specific δ^{13}C of *n*-alkanes during the drought phase I, suggests that grassland and heathland shoots were actively synthesizing long-chain *n*-alkanes in order to withstand drought.

A comparison of grassland and heathland plant-soil systems highlighted differences in *n*-alkanes compound-specific δ^{13}C values especially of their root systems, which revealed the different adaptation mechanisms of both ecosystems under drought. Finally, the observed changes in the soil *n*-alkane composition, especially during the first 40 days of drought, point to a subsequent incorporation of shoot and root-derived alkanes under drought. The outcome of this study provides valuable information about the acclimatization of plants during drought phase I.

3.3 Manuscript III: Plant C uptake, its assimilation and translocation into soil decreases with an increase in the duration of drought (Srivastava *et al.*, 2017b)

The research question 3 of this thesis (question 3, subsection 1.3) was asked to understand the potential impact of increasing drought duration on C uptake by plants and C translocation into the soil in model grassland and heathland ecosystems that have been subjected to a severe drought and irrigation.

To investigate the short-term C dynamics of freshly assimilated photosynthetic C, a conceptual approach included three $^{13}CO_2$ pulse-chase labelling experiments of plants that were exposed to different phases of a severe drought (Figure 2.3) in order to trace above- and belowground C allocation patterns. This is the most interesting and unique feature of this study. These experiments comprised of $^{13}CO_2$ pulse-chase labelling grassland and heathland plant communities in the field at three different phases of the 104 days of drought. The first isotopic pulse label was applied on days 0/1, the second on days 44/45, and the third on days 70/71. The labelling procedure in detail is described in Manuscript III. Additionally, the re-allocation of C taken up by the plants during the drought period and its incorporation into the soil after the irrigation phase was monitored in the plant-soil system of both ecosystems.

Shoots, roots and soil samples were analysed for their ^{13}C excess in grassland and heathland ecosystems. The results for freshly assimilated ^{13}C demonstrated that among two grassland and two heathland plant species, respectively (*P. lanceolata* and *H.lanatus*) and (*V. myrtillus* and *C. vulgaris*), all plants displayed the highest ^{13}C tracer uptake during the first labelling (Figure 3.4) compared to the other pulse labellings. After the first 40 days of drought all plant species showed lower ^{13}C tracer uptake which is in agreement with the literature (Fuchslueger *et al.*, 2014; Hasibeder *et al.*, 2015). No further C uptake was observed after 70 days for the remainder of the drought. The observed reduction in ^{13}C excess after 70 days of the drought could not be verified by other studies due to its very long duration, which is uncommon in other field studies

on drought manipulation.

For heathland roots, the ^{13}C excess showed that after the first pulse during drought phase I, C was actively transported from shoots to the root systems. This indicated that for heathland plants a comparatively large amount of assimilated C was allocated towards roots, which is in agreement with the literature (Carbone & Trumbore, 2007). In contrast to heathland, grassland roots revealed the maximum ^{13}C excess 40 days after the first labelling. This ^{13}C allocation pattern in the grassland plant-soil system confirmed the slower translocation due to drought (de Vries *et al.*, 2016). The time-lag for C allocation from shoots towards roots in grassland was expected to be shorter compared to the heathland plants during the first labelling because the speed of C allocation depends on the plant size (Kurtz Jr., 1950). This indicated that the ^{13}C was utilized for the maintenance of shoots and growth of roots under drought (Hasibeder *et al.*, 2015). After the second and third pulse labelling, heathland roots revealed comparatively slower C allocation dynamics than grassland roots, which argues for the conservative nature of perennial heathland plants (Carbone & Trumbore, 2007).

Similar to shoots and roots, ^{13}C excess for grassland and heathland soil demonstrated that with an increase in drought duration, less C is incorporated into the soil via roots as observed in the first and second labelling. After the third pulse labelling, the data exhibited remarkably no ^{13}C input in the soil. Shoots, roots and soil samples that were labelled during the second and third pulse labelling campaign and collected during the irrigation phase showed a remarkable result. Interestingly, 5–10% relative allocation of assimilated ^{13}C was observed in roots vs. shoots for both ecosystems. This is associated with the re-activation of C allocation from shoots to roots and its incorporation into the soil.

To conclude, extreme drought may lead to a reduction in the C uptake and allocation by plants as well as its incorporation into the soil for both ecosystems. Furthermore, the result of irrigation on ^{13}C tracer signal in shoots, roots and soil (highlighted in grey colour (Figure 3.4)) demonstrated that the labelled ^{13}C, that was assimilated in shoots during the drought period, was re-allocated towards roots and soils for both ecosystems only within 10–15 days.

This study demonstrated that severe drought followed by irrigation clearly changed the C uptake by shoots, its assimilation within the shoots, the belowground C allocation and its incorporation into the soil. In summary, this study provides clear evidence that with an increase in the drought duration, plant C uptake and its translocation into the soil may be seriously affected, which alters the quantity and quality of plant C inputs for microbial decomposition and thus affects the overall C turnover and storage in soil.

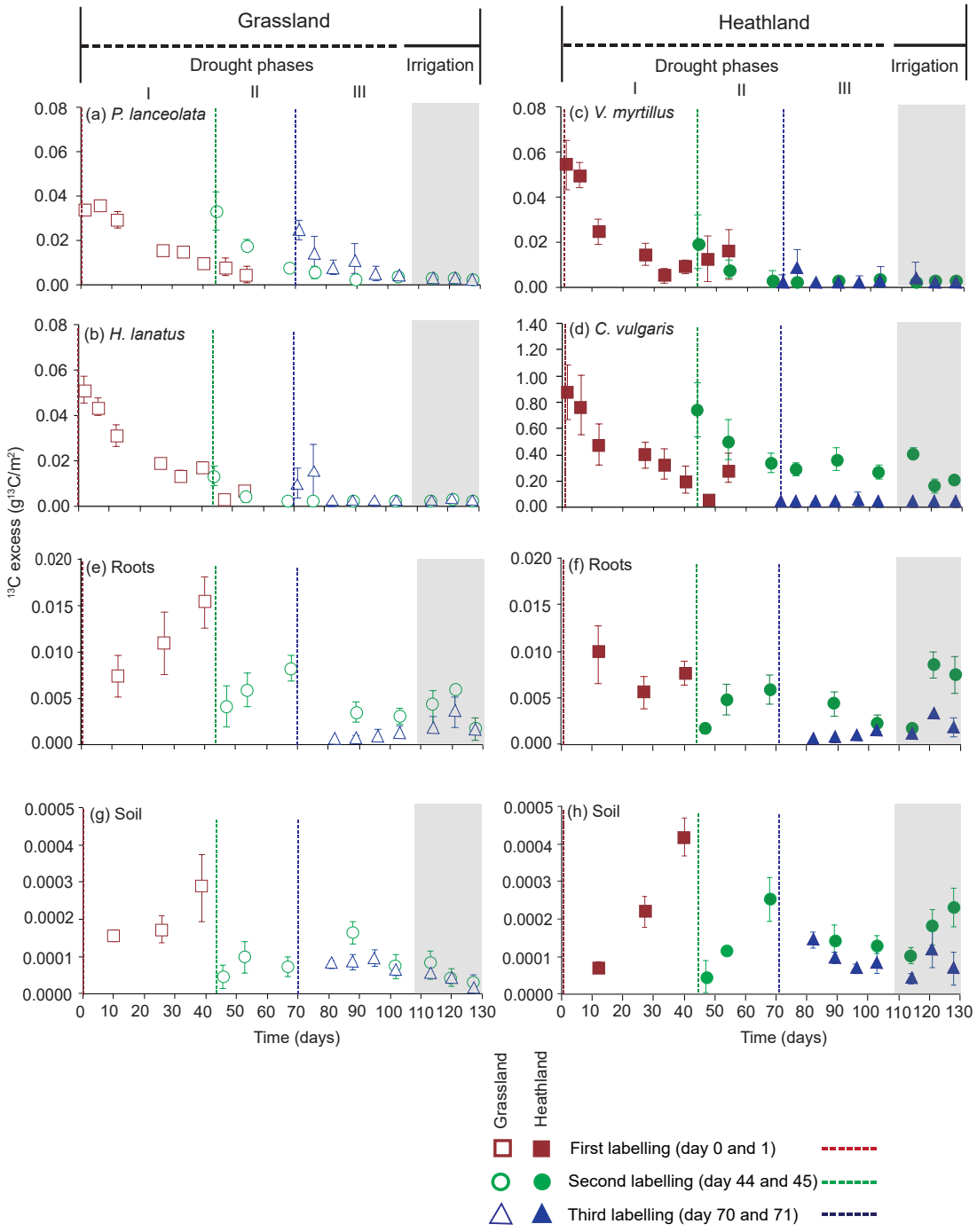

Figure 3.4: Plant C uptake and input in soil under different phases of drought and irrigation. (a–d) ^{13}C excess (g/m^2) in shoots, (e–f) roots and (g–h) soils of a model grassland and heathland ecosystem. $^{13}CO_2$ pulse labelling is indicated by dotted line three times for day 0/1, 44/45 and 70/71 in grassland and heathland respectively. Mean and standard errors of mean are given (field replicates, n = 6). Periods of irrigation treatments are shaded in grey and highlighted by solid line.

4 Conclusions

This thesis contributes to a general understanding of C cycling (at a molecular level) in grassland and heathland ecosystems during a severe drought followed by irrigation. The conclusions of each research question (as mentioned in subsection 1.3) are summarized below.

Question 1.: What is the effect of five years of repeated annual drought (100 year extreme) on the C and lipid compositions in grassland and heathland plant–soil systems?

(i) Contrary to the expectation, perennial heathland plants were not significantly influenced by repeated annual drought, i.e. the plants have no 'memory' effect in terms of their biosynthesis mechanisms.

(ii) Surprisingly, no significant changes in soil C and lipid concentrations were observed after five years of repeated annual drought.

Question 2.: How does a severe drought affect lipids composition (*n*-alkanes) in the plant-soil systems in grassland and heathland communities?

(i) In agreement with the hypothesis, increased TLE concentration and *n*-alkanes chain length in shoots and roots demonstrated a direct input of plant-derived long-chain *n*-alkanes in soil. Increased CSIA of *n*-alkanes (2–3‰) in shoots during the drought phase I indicated that C was actively utilizing for *n*-alkane biosynthesis in order to withstand drought. Furthermore, unmodified values of δ^{13}C of *n*-alkanes in soil revealed that plant–derived *n*-alkanes could not be determined.

(ii) As expected, higher concentrations of TLE and *n*-alkanes were determined in heathland vs. grassland plants, demonstrating a higher resistance of heathland plants against water stress. The investigated grassland and heathland plants have different strategies to adapt their lipid biosynthesis during drought.

Question 3.: What is the impact of drought on the short–term C dynamics in the plant-soil system of grassland and heathland ecosystems and how do these ecosystems respond to irrigation after drought?

(i) As hypothesised, C uptake by plants and its translocation towards the soil decreased with increase in drought period. Compared to the first pulse labelling during the first drought phase, significantly lower uptake of C in plant biomass and C incorporation in soil was observed during the second pulse labelling. After 70 days, plant C uptake and its allocation towards the soil significantly reduced with the increasing duration of the drought.

(ii) Interestingly, 10–15 days after irrigation C that was stored in the shoots during the second and third isotope pulse, was re-allocated into roots and soil. This demonstrates a fast ecosystem recovery in terms of re-activation of C cycling in the plant-soil system.

5 Implications of the thesis in a wider context

Climate change is causing measurable changes in the precipitation pattern across Central Europe which could have uncertain implications for the processes involved in the C cycling in the temperate grassland ecosystem. Field studies focusing on the severe effects of drought on temperate grassland and heathland ecosystems to establish links between above- and belowground metabolic processes are lacking till date. This thesis contributes to fill this knowledge gap. The different extremes and durations of applied drought phases followed by irrigation treatments were very fruitful for testing the resistance of temperate grassland and heathland ecosystems. The results presented in this thesis are of great interest for the mechanistic understanding of the impact of drought in both ecosystems. It can thus help to verify the results of short or long-term laboratory studies on drought that investigate C and lipid dynamics in temperate ecosystems, though caution is required in comparing field and laboratory experiments. In addition, the key message of this study could not be simply transferred to other natural temperate ecosystems, though it could surely be applicable up to a certain level.

A graphical summary of the results discussed in this thesis is shown in Figure 5.1.

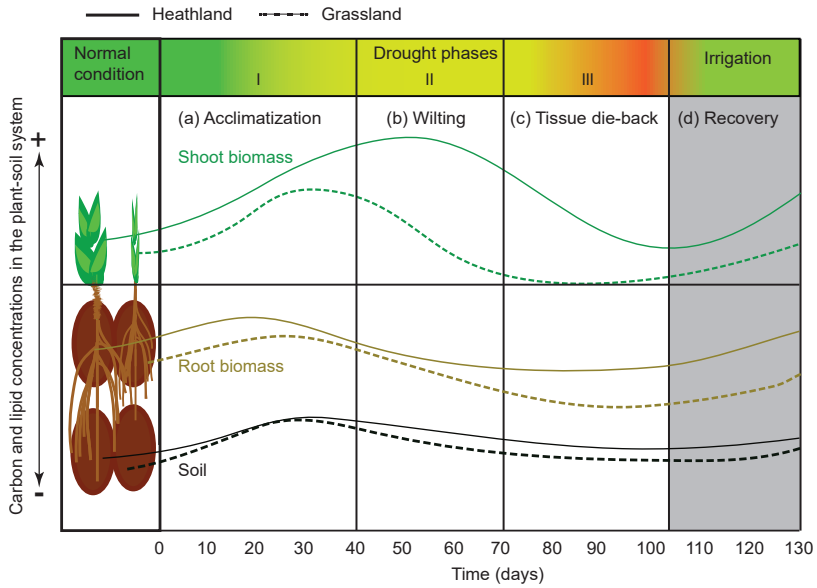

Figure 5.1: Summary of plants metabolic response to different drought phases. This diagram is based on the drought stress response of a model ecosystem as suggested by Walter *et al.* (2013); Backhaus *et al.* (2014) and results obtained in Manuscript II and III. Dashed line represent grassland whereas solid line for heathland plants. Green, yellow and red colour indicate healthy, drier and dead conditions of the aboveground biomass respectively. Change in the growth condition of plants from normal to a severe drought followed by irrigation, stimulated several metabolic changes at a cost of C investment. These changes can be described mainly by 4 processes. (a) Acclimatization (drought phase I), where photosynthesis was not limited and therefore, more lipids were produced in shoots and roots in response to a drought period. During this phase, plant-derived lipids were incorporated into soil. (b) Wilting (drought phase II) when C uptake was limited in shoots, but belowground C translocation remain unaffected. (c) Tissue-die back (drought phase III), when plants started to get dry from aboveground part, whereas roots were still remained alive. (d) Recovery of plants after irrigation, which rapidly regenerate and restart C cycling in the plant-soil system. These findings revealed that shoots prioritize the C investment especially towards roots to regain root functions after irrigation.

The plant metabolic response under drought is an important factor that strongly affects the survival of plants. During the beginning of the drought stress (phase I), the plants acclimatized to the drought and adapted to the stressed period with a rapid biosynthesis of *n*-alkanes (Shepherd & Griffiths, 2006; Sachse *et al.*, 2015) at a cost of C investment as suggested by Kleczewski *et al.* (2010) and in agreement with the literature (Brüggemann *et al.*, 2011; Fuchslueger *et al.*, 2014). The results of the Manuscript II show that the investigated plants quickly respond to drought. This is why an increase in lipid composition and *n*-alkanes was observed only during the drought phase I (0–40 days). Also for the soil, during the drought phase I, the incorporation of plant-derived *n*-alkanes was observed in both ecosystems, indicating that soil C storage can increase in the beginning of the drought but not in the later drought phases II and III. However, the continuous production of *n*-alkanes within the plant during drought phases II could be an adaptive mechanism for maintaining the vitality of the plants (Harb *et al.*, 2010; Sachse *et al.*, 2015; Yan *et al.*, 2016). Therefore, the absence of changes in lipid composition during drought phases II and III suggest that the continued production of long-chain *n*-alkanes in plants is not necessarily only a possible aspect of the adaptive mechanism in plants to drought (Shepherd & Griffiths, 2006).

However, this thesis could not draw any general conclusion about the drought sensitivity or drought tolerance of plants based on their C and lipid composition, because all investigated plants (especially their roots) survived under severe drought. This is clearly shown by the results of the pulse labelling experiment in Manuscript III. A synthesis of results from manuscripts II and III indicates that plant metabolic responses were faster in grassland compared to heathland plants. This indicates that the ability of grassland plants to adapt to drought is relatively faster than for heathland plants, which nevertheless survive and maintain their vitality longer under drought (Yan *et al.*, 2016).

The first labelling results revealed that the ^{13}C tracer uptake by shoots and its allocation towards roots was very fast in heathland compared to grassland. Plant C uptake was reduced significantly after 70 days of drought and by consequence the supply of C belowground also decreased for both ecosystems, which is in agreement with literature (Burri *et al.*, 2014; Fuchslueger *et al.*, 2014). A close coupling of above- and belowground C cycling clarified a distinguished allocation pattern of C from shoots to roots in grassland and heathland ecosystems during the drought phase I. This means that drought response cannot be simply generalized across ecosystems by either investigating only aboveground or belowground biomass.

In terms of C assimilation and allocation, heathland plants exhibited higher amounts than grassland. This is associated with their well developed root systems (Carbone & Trumbore, 2007). The result of irrigation followed by drought periods demonstrated that a re-allocation of C in roots and soil occurred for both ecosystems. This indicates a re-activation of C cycling in the plant-soil system even after a strong drought (Xu & Zhou, 2007; Xu *et al.*, 2010). This suggests that only shoots were dried or died during drought phase III, but roots were still alive throughout the drought period. This is why C cycling could be re-activated as soon as irrigation started (Zwicke *et al.*, 2015).

Furthermore, the re-allocation of C in roots and soil after irrigation confirmed no long-term impact of drought in the plant-soil system. This is inconsistent with the literature (Lei *et al.*, 2016). Nevertheless, the results are in agreement with Backhaus *et al.* (2014) who suggested that recurrent drought might increase plant resistance and observed no long-term influence under severe drought. Hence, this thesis summarizes that the projected climate change might have no significant impact on the long-term lipid dynamics and C-budget in the plant-soil system of temperate ecosystems. However, it is important to take into account that not all droughts have an equal impact on C cycling (Hoover, 2014), because photosynthesis and C allocation in plants are partially determined based on the intensity of the drought conditions (Chaves *et al.*, 2003; Yan *et al.*, 2016). This is supported by many studies (Jentsch *et al.*, 2011; Reichstein *et al.*, 2013; Hasibeder *et al.*, 2015).

Hence, the studies revealed that a long-term influence of drought on the C dynamics in the plant-soil system might be strongly influenced by the experimental design and drought-monitoring period (Hoover, 2014; Gilbert & Medina, 2016). Furthermore, different soil and different ecosystem types with different plant species and plant ages, time scales, nutrient levels and water holding capacities of the soil will have the potential to bias the results determined in this thesis. Therefore, similar approaches on other sites and in other ecosystems are strongly required to test the generality of the findings of this thesis.

6 Perspectives

In the context of global climate change, the role of lipid bio-marker has recently received a considerable interest due to its importance to protect plants against drought and also considered as an intermediate stable C-pool in soil. This study revealed a quick response of plant-derived *n*-alkane composition and its fast incorporation into the soil under drought. However, the compound-specific isotope composition of *n*-alkanes in soil did not change significantly throughout the drought period. This result is surprising and would benefit from further investigation. Therefore, fatty acids which are a precursor of *n*-alkanes in plants or other related compound classes such as alcohols or aldehydes could verify if the applied drought scenario actually stimulated the formation and translocation of long-chain *n*-alkanes compounds in the temperate grassland and heathland plant-soil systems or not. Hence, in order to find a close coupling between plant-derived *n*-alkanes and its fate in soil, further research is required to answer the following questions:

1. What is the fate of long-chain fatty acids (which are the main precursors for the formation of *n*-alkanes) in temperate grassland and heathland ecosystems, when they are exposed to severe drought?

2. What is the response of the plant-soil system to irrigation after a severe drought event in terms of the lipid composition (including fatty acids and *n*-alkanes)?

Together with current CSIA for δ^{13}C *n*-alkane data, fatty acid δ^{13}C data may allow a better understanding of the relative contributions of plant-derived C input in soil under drought and irrigation. The CSIA analysis of fatty acids together with *n*-alkanes will enable estimating the proportions of newly synthesized compounds in plants as well as the translocation and mineralization in soil during severe drought. Combining the results from fatty acids and *n*-alkanes will improve our understanding of the role of plant-derived lipids and their significance for soil C storage under drought.

Furthermore, apart from CSIA for δ^{13}C, hydrogen isotope composition (δ^{2}H) of plant waxes (*n*–alkanes and fatty acids) could enable obtaining valuable information about the efficiency of plant water-use and hydrological conditions or plant-water status during droughts.

Moreover, some other compounds such as lignin (the second most abundant polymer), which are not directly associated with plant-water relation and relatively resistant to degradation, can provide further insights into other structural compound classes and their fate in plant-soil systems that experience severe drought due to the ongoing-global climate change.

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Part B

Manuscripts

Manuscript I

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Authors	Contributions
Kavita Srivastava (50%)	Sample collection, sample preparation for lipid analysis and bulk elemental and isotopic analysis. Responsible for the execution of the data acquisition, statistical analysis of data and their illustration in tables and figures. The manuscript is written together with the contribution of all co-authors.
Anke Jentsch (5%)	Main initiator of the Event experiment, where samples were collected.
Bruno Glaser (15%)	Conducted bulk elemental (C, N) and stable isotopic (δ^{13}C, δ^{15}N) analysis (EA-IRMS) of shoots, roots and soil samples.
Guido L.B. Wiesenberg (30%)	Concept and idea of the project. Proposed and supervised the study.

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Repeated annual drought has minor long-term influence on $\delta^{13}C$ and alkane composition of plant and soil in model grassland and heathland ecosystems

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Abstract

As a result of global climate change the incidence of drought conditions in Europe is predicted to increase in the future, which also influences plant resistance. Lipids are important plant constituents that protect plants against drought stress and contribute to the intermediate stable carbon (C) pool in soil. However, the extent to which drought influences lipid cycling in the plant–soil system is unknown and, therefore, it remains questionable how the ecosystem recovers after drought. We focused on plant and soil samples from two different plant communities (temperate grassland and heathland) that had been exposed to 5 years of 4.5–6.0 weeks repeated annual drought. They were sampled one year after the last drought to check the recovery of the plant–soil system. Samples were analyzed for their bulk C, stable C and nitrogen (N) isotope ($\delta^{13}C$, $\delta^{15}N$) and lipid composition. Contrary to our expectation, no strong influence of five years of repeated annual drought was observed for above-ground biomass, roots and soils in the model ecosystems with respect to elemental (C and N concentrations, C : N ratio) bulk isotope ($\delta^{13}C$, $\delta^{15}N$) composition and the total extractable lipid concentration. Thus, plants did not sustain a significant change in their C and lipid concentration as well as their composition after five years of repeated annual drought. This might be related to the comparatively short drought period related to the overall growth season and provides evidence for recovery of the C and lipid dynamics in temperate grassland and heathland model ecosystems exposed to annual drought.

Key words: average chain length (ACL) / carbon preference index (CPI) / EVENT experiment / lipid composition

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1 Introduction

Global climate change is predicted to significantly change global precipitation patterns, leading to extreme weather events, such as drought periods, which might increase in intensity and frequency (*Ciais* et al., 2013). To understand the response of terrestrial ecosystems to changing precipitation patterns, the influence of drought on the plant–soil system has been studied worldwide (*Jentsch* et al., 2007; *Jentsch* and *Beierkuhnlein*, 2010; *Smith*, 2011). Drought reduces plant growth (*Ciais* et al., 2005; *Zhao* and *Running*, 2010), C cycling, and C storage in the plant–soil system due to a reduction in photosynthesis rate (*Chaves* et al., 2002; *Jentsch* and *Beierkuhnlein*, 2008). Repeated annual drought experiments revealed that a single drought event (100 year extreme) can alter C fluxes in grasslands (*Mirzaei* et al., 2008; *Jentsch* et al., 2011), even without significantly altering above-ground as well as below-ground biomass production (*Kreyling* et al., 2008a; *Kreyling* et al., 2008b; *Jentsch* et al., 2011). However, the relationship between above-ground biomass C uptake, translocation of C within plants towards roots and soil, as well as C cycling in the plant–soil system are rarely addressed in drought stress experiments. Further, the adaptability of the ecosystems is not yet clearly understood and it remains questionable how plant–soil systems recover after repeated annual drought.

In response to drought, plants exhibit drought resistance mechanisms, which are classified into drought avoidance (maintenance of tissue water potential) and drought tolerance (*Jones*, 2007). Epicuticular waxes provide a protective coating on plant leaves and stems, which has an important physiological and ecological function in the interaction of plants with their environment (*Eglinton* and *Hamilton*, 1967; *Jetter* et al., 2006), such as protection against water loss. This is promoted by the hydrophobic constituents of the cuticle, such as cutin and other lipid components such as long-chain *n*-alkanes, wax esters and other long-chain alkyl compounds (*Kolattukudy*, 1970; *Harwood* and *Russell*, 1984). In theory, plants might be able to change their cuticular composition in response to drought, *i.e.*, to improve the hydrophobicity of the wax layer by producing a higher amount of alkyl lipids (*Shepherd* and *Griffiths*, 2006). As lipid biosynthesis has been observed to respond quickly to environmental change (*Jenks* et al., 2001), this ability depends upon the plant species

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(*Peñuelas* and *Llusià*, 2003). In particular, *n*-alkanes, as abundant compounds in epicuticular wax, are thought to regulate diffusion of water inside and outside of leaves (*Eglinton* et al., 1962), which is especially crucial under drought stress. Furthermore, alkanes, largely contributing to the neutral lipid content in plant leaves, were observed to increase significantly under drought conditions (*Hamrouni* et al., 2001). However, most of these findings related to changes in lipid metabolism as a response to drought rely on laboratory or short-term field experiments, while the sustainable effect of drought on plant lipid composition was not investigated. Hence, it remains unclear if plant tissues sustain a change in their lipid composition when exposed to repeated annual drought in order to protect themselves against drought or if they are resistant in the way that no long-term effects in the wax quantity and composition are preserved after drought.

The influence of drought is also very important to understand the stability of soil organic matter under changing environmental conditions. Carbon storage in soils has been widely investigated during past decades from various perspectives including chemical fractions (*Kögel-Knabner*, 2002), yielding only a few chemical fractions that were characterized by higher stability than bulk organic matter (*Marschner* et al., 2008). One of these fractions was identified as lipids, of which alkanes as hydrophobic compounds in soil were characterized by slower turnover than that of bulk C (*Cayet* and *Lichtfouse*, 2001; *Marschner* et al., 2008; *Schmidt* et al., 2011).

The main sources for soil lipids are higher plant-derived abraded leaf wax (*Conte* et al., 2003), litterfall, and rhizodeposition (*Wiesenberg* et al., 2010). In addition, microorganisms can contribute a considerable proportion to soil lipids and, furthermore, they contribute to degradation of plant-derived lipids (*Harwood* and *Russell*, 1984; *Wiesenberg* et al., 2010). Although plant-derived lipids like *n*-alkanes can be relatively stable in soil (*Marschner* et al., 2008), the influence of drought on soil lipid composition has not been investigated. Hence, it remains unclear whether incorporation of plant-derived lipids is limited under drought conditions and how storage and turnover of lipids in soil is subsequently affected. Although alkanes are not largely abundant in soils, they contribute to the slow reacting C pool and are therefore of great relevance for studying the impact of drought on C cycling in the plant–soil system. This might be of greater relevance in temperate ecosystems, if droughts occur more frequently in the future and the hypothesis holds true that stability of soil organic matter is an ecosystem property (*Schmidt* et al., 2011) and depends on environmental factors including drought.

The main goal of this study was to investigate the effect of repeated annual drought on C and lipid composition in a plant–soil system under field conditions as annual recurrent drought is a feature of many temperate ecosystems and is predicted to increase in frequency and duration in the future. Thus, it is important to determine the effect of long-term drought extremes (*i.e.*, > 100 years) in order to understand future ecosystem response in terms of bulk C and lipid composition. We focused on model grassland and heathland plant–soil systems that were annually exposed to a drought period of 4.5–6.0 weeks, without rainfall in spring from 2005 to 2010, in the Bayreuth EVENT I experiment (*Jentsch* et al., 2007; *Jentsch* et al., 2011). We hypothesized that (1) the annual grassland plants in these systems do not sustain a change of their lipid composition when exposed to annual drought, as opposed to perennial heathland plants, as only the latter have the capability of adapting to changing environmental conditions during their lifetime, and (2) one year after the five years repeated annual drought, the soil C concentration would be expected to decrease, whereas lipids increase relative to the C concentration. This is expected to be due to reduced incorporation of biomass into soil under drought, followed by preferential degradation of readily available substrates such as sugars, leading to preferential enrichment in less degradable compounds such as lipids.

2 Material and methods

2.1 Site description

The current study is a part of the EVENT I experiment at the Ecological-Botanical Garden of the University of Bayreuth, Bayreuth, Germany (49°55′19′′ N, 11°34′55′′ E, 365 m asl) that focused on the impact of extreme climate events on model grassland and heathland ecosystems (*Jentsch* et al., 2007; *Jentsch* et al., 2011). In this experiment, extreme events such as 100 to 1000 year drought and heavy rain events as well as increased freezing-thawing cycling have been annually applied and compared to control conditions. After five years, the drought plots revealed significant differences compared to control plots in terms of the plant biodiversity (*Jentsch* et al., 2011); therefore we chose these for sample collection. The mean annual air temperature and annual precipitation at the site were 8.2°C and 724 mm, respectively. During preparation of the experimental site, the upper soil (0.0–0.2 m depth) was produced from homogenized topsoil from a nearby quarry distributed on homogenized sand from the same quarry (0.2–0.8 m depth), underlain by drainage to avoid soil-related anomaly. The initial texture of the soil was loamy sand (820 g kg^{-1} sand, 130 g kg^{-1} silt, 50 g kg^{-1} clay). In the upper soil layer, pH was 4.5 (measured in 1 M KCl) and in the lower layer it was 6.2.

The EVENT I experiment was set up in a multifactorial design, investigating several plant communities with different biodiversity levels combined with different environmental manipulations, *i.e.*, drought, heavy rainfall, and freeze-thaw cycle, which could be compared with control plots. The setup was based on a latin square design consisting of 150 plots, with a size of 2 m × 2 m per plot with every factorial combination replicated five times. For the current study, we chose plots manipulated *via* two factors: (1) change in precipitation (expected 100-year extreme drought event and control) and (2) plant species variation (grassland and heathland plant communities). The plant species composition used in the experiment consists of frequently occurring plant species on sandy soils across Central Europe (*Lindborg*, 2007). A limited number of species was selected for the EVENT I experiment with respect to their common occurrence, their affiliation to defined functional groups such as grasses, forbs/legumes and dwarf

shrub/woody species, and with respect to life-span, *i.e*., from annual to perennial (*Jentsch* et al., 2007; *Jentsch* et al., 2011). The plant community of the grassland plots was initially established with *Plantago lanceolata* (*P. lanceolata*), *Holcus lanatus* (*H. lanatus*), *Lotus corniculatus* (*L. corniculatus*), and *Arrhenatherum elatius* (*A. elatius*). As *A. elatius* disappeared from some of the plots during the experiment, we focused only on the three other grassland species. In the heathland, a community of *Calluna vulgaris* (*C. vulgaris*) and *Vaccinium myrtillus* (*V. myrtillus*) was chosen.

Five independent replicates were available for all plots with different treatments and plant communities within the EVENT I experiment. A five-year repeated annual drought treatment was applied during the most active period of the growing season for most of the plants (May–July), whereas the typical length of the growth season for Northern Bavaria is March until September (*Jentsch* et al., 2011). Due to different phenology of the investigated plant species and local climate conditions (*Automatic weather station*, 2017; Botanical Garden Bayreuth) the most active part of the growth season at the EVENT site is between April and August, which accounts for approximately 21.5 weeks in accordance with *Menzel* (2003). The intensity of drought was based on the local 100-year and 1000-year extreme weather events (*Jentsch* et al., 2007). For drought manipulation, rainout shelters (6 m × 8 m, center height 3 m) were established for 4.5 and 6 weeks each year in May for 2005–2007 (100-year extreme) and 2008–2010 (1000-year extreme), respectively. These periods were equivalent to ca. 21% and 28% of the most active part of the growth season. The rainout shelters were constructed with a steel frame and covered with a transparent plastic sheet, which permitted nearly 90% penetration of photosynthetically active radiation. Greenhouse effects due to rainout shelters were avoided by installing the roof at a height of 80 cm, enabling near-surface air exchange (*Jentsch* and *Beierkuhnlein*, 2008). To prevent lateral surface flow of water from the roof, plastic sheet pilings were inserted 10 cm into the soil surrounding drought-treated plots, which emerged from the soil for another 10 cm. The control plots were maintained by regular irrigation using a portable irrigation system if the precipitation remained below the common annual precipitation level, to avoid drought periods.

2.2 Sample collection and preparation

Plant and soil samples were collected from the selected 20 plots in May 2011, *i.e*., one year after the last drought, to identify the resilience of the plant–soil systems in terms of lipid and bulk elemental (C, N) composition after five years of repeated annual drought. Due to instrumentation of the plots and ongoing experiments, only one quarter of each plot was available for sampling. In each quarter, sampling was performed at least 10 cm away from any margin in order to avoid edge effects. From each plot, shoots of several plant individuals were collected accounting for at least 15 leaves or a few twigs with green leaves per plant species, which were pooled for each individual plant species and analyzed separately for each plot. For soil samples, an auger (15 cm × 5 cm inner diameter) was used, which was introduced three times per plot, the soil aliquots being combined. All shoot and soil samples were oven-dried at 40°C. Roots were removed from soil samples and collected separately with tweezers, washed with de-ionized water, and oven-dried at 40°C. Unfortunately, it was not possible to collect roots separately for individual plant species. Hence, grassland roots represent the mixture of roots from the four plants species, whereas heathland roots were a mixture of two heathland species. Soil samples were dry-sieved to a particle size < 2 mm. All samples were ground in a ball mill (Retsch MM 200, Germany) to fine powder.

2.3 Elemental (C, N) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) analysis

Soil (10 mg), shoot, and root samples (1 mg each) were weighed in Sn capsules to determine C and N concentrations, as well as stable C ($\delta^{13}C$) and N isotope ($\delta^{15}N$) composition. Measurements were performed using an elemental analyzer (Eurovector, Milan, Italy) coupled to a ConFlow III interface (Thermo Fisher, Bremen, Germany). Combustion of samples was followed by gas chromatography (GC) separation and transferring to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany). To calculate stable C and N δ values, the measured ^{13}C abundance and ^{15}N abundance values were normalized to the V-PDB (Vienna Peedee Belemnite) standard using international reference materials (International Atomic Energy Agency, Vienna) for calibration: IAEA-350, IAEA-CH-6, IAEA-CH-7, IAEA-N-2, IAEA-NO-3, and USGS 41. All standards were measured repeatedly within a sample measurement sequence. Isotope ratio values ($\delta^{13}C$) are expressed as per mil δ (‰) values relative to the V-PDB standard and those of N ($\delta^{15}N$) to the N_2 of air:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[\left(R_{(sample)}/R_{(standard)} - 1\right)\right] \times 10^3, \quad (1)$$

where $R_{(sample)}$ is the ratio of the heavy isotope (^{13}C or ^{15}N) to the respective light isotope (^{12}C or ^{14}N) of the sample and the respective standard, with $R_{(standard)}$ = 0.0111802 for V-PDB and $R_{(standard)}$ = 0.003676 for air. Total C concentration and $\delta^{13}C$ values correspond to organic C, since carbonate C was absent from soil and plant samples. Unfortunately, heathland root samples from the drought plots could not be analyzed for their elemental and isotope composition due to low sample amount and analytical problems.

2.4 Expected change of C in soil based on stable isotope ($\delta^{13}C$)

To determine the theoretical annual change in soil $\delta^{13}C$, contribution of plant-derived $\delta^{13}C$ was required. However, due to sample unavailability for the period 2005–2010, we used changes in $\delta^{13}C$ isotope values that were recorded for the same plant species during another field experiment that was carried out at the same site in Bayreuth in 2011 (unpublished data). The expected change in $\delta^{13}C$ in plants had to be extrapolated to the most active part of the growth season April–August (*Menzel*, 2003), assuming that during the drought period biosynthetic isotope fractionation at the time of biomass production led to a change in $\delta^{13}C$ values, whereas before and thereafter this change was negligible. Therefore,

the isotopic shift for the years 2005–2007 (4.5 weeks) contributed 21% to the end-of-the season plant isotope signal and for the years 2008–2010 (6 weeks) 28%, respectively. Contribution of different species to the plant-derived isotope signal entering the soil can be estimated based on the relative contribution of biomass of the individual species (*Jentsch* et al., 2011), determined at the end of the different growth seasons by calculating weighted averages of the $\delta^{13}C$ values of the shoot biomass. Considering root-to-shoot ratios also the relative contribution of root and shoot biomass was taken into account. Hence, plant-derived changes of $\delta^{13}C$ were calculated using the following equation:

$$\Delta^{13}C_{Plant} = (\Delta^{13}C_S \times (1 - R/S)) + (\Delta^{13}C_R \times R/S), \quad (2)$$

where $\Delta^{13}C$ is the difference between $\delta^{13}C$ values under drought *vs.* control treatments and *S* and *R* represent the dry biomass (g m^{-2} y^{-1}) determined for shoots and roots, respectively. Root biomass represents a mixture of roots of four plants for grassland and two plants for heathland. For grassland and heathland plants, shoot biomass represents the weighted average of the isotopic differences for two plant species. The calculated annual contribution of plant-derived isotope difference ($\Delta^{13}C_S$ and $\Delta^{13}C_R$) corresponds to the drought period of 4.5 weeks in years 2005–2007 and 6 weeks in years 2008–2010. Due to the fast turnover of fresh plant biomass and successive incorporation of plant-derived organic matter into aggregates (*Dorodnikov* et al., 2011), an annual change of 10% was assumed for the short duration of the experiment:

$$\Delta^{13}C_{Soil} = \sum (0.1 \times \Delta^{13}C_{Plant})_i, \quad (3)$$

where, $\Delta^{13}C_{Soil}$ is the expected change in $\delta^{13}C$ values based on drought-induced isotope shifts of plant $\delta^{13}C$ values for grassland and heathland soils under drought *vs.* control after 5 years of repeated annual drought using *i* as the respective values for the years 2005 until 2010.

2.5 Lipid analysis

Shoot, root (0.5–2.0 g), and soil (40–50 g) samples were extracted *via* Soxhlet extraction for at least 36 h using CH_2Cl_2/MeOH (93 : 7; v/v; *Wiesenberg* et al., 2004). After evaporation of the solvent, the total lipid extract was sequentially separated into three fractions based on polarity. Neutral lipid and fatty acid (FA) fractions were obtained using solid-phase extraction (SPE) with KOH-coated silica gel (*Wiesenberg* et al., 2004). Neutral lipids were eluted with CH_2Cl_2, followed by FA, which were recovered with CH_2Cl_2/HCOOH (99 : 1; v/v). Neutral lipids were then further separated into aliphatic, aromatic and low polarity hetero compounds using a column filled with activated silica gel (10 nm). Aliphatic hydrocarbons were eluted with hexane followed by aromatic hydrocarbons with hexane/CH_2Cl_2 (1 : 1; v/v), and low polarity hetero compounds with CH_2Cl_2/MeOH (93 : 7; v/v). Volume reduction was performed *via* rotary evaporation. These different steps yielded five lipid fractions, of which only aliphatic hydrocarbons as representatives for hydrophobic and the most persistent plant and soil lipids were studied in detail.

A defined amount of a deuterated *n*-alkane standard (D_{50}-*n*-C_{24}) was added to the individual aliphatic hydrocarbon fractions prior to gas chromatographic (GC) analysis for quantification purposes. Compound assignment was performed using a GC instrument equipped with splitless injector and flame ionization detection (GC-FID; Agilent 7890) and comparison with a standard mixture. A J&W HP 5 column (30 m × 0.32 mm, 0.25 μm film thickness) was used with He (purity 5.0) as carrier gas at 2 mL min^{-1}. After 2 min at static temperature in the beginning, the GC oven was programmed from 70°C (2 min) to 320°C (held 20 min) at 5°C min^{-1}.

2.6 Alkane molecular proxies

The average chain length (ACL) of aliphatic hydrocarbons in soil is used to differentiate different sources of organic matter (OM):

$$ACL = \sum(Z_n \times n) / \sum(Z_n), \quad (4)$$

where *n* is the number of carbons and Z_n the amount of alkanes with *n* carbons, with *n* in the range $C_{17–33,}$ used for ACL. High ACL values (≥ 27) derive from higher plant biomass, which is dominated by long-chain *n*-alkanes ($C_{25–33}$; *Eglinton* et al., 1962; *Kolattukudy*, 1970). In contrast, the contribution from short-chain *n*-alkanes ($C_{15–23}$), deriving mainly from microbial biomass and degradation processes, leads to low ACL values (≤ 27; *Harwood* and *Russell*, 1984).

Higher plant biomass is typically enriched in odd *n*-alkanes (*Eglinton* et al., 1962; *Kolattukudy*, 1970), which can be expressed *via* the carbon preference index (CPI):

$$CPI = \left[\left(\sum C_{23-33\text{odd}} / \sum C_{22-32\text{ even}}\right) + \left(\sum C_{23-33\text{odd}} / \sum C_{24-34\text{even}}\right)\right] / 2 \quad (5)$$

High values (> 10) are typical for fresh leaf biomass, while values close to 1 indicate strong degradation of OM (*Cranwell*, 1981). Furthermore, values around 1 are characteristic for microorganism-derived OM, because the latter comprises mainly short-chain *n*-alkanes without even or odd predominance (*Cranwell*, 1981).

Long-chain *n*-alkane distribution patterns dominated by *n*-C_{27} or *n*-C_{29} are often enriched in shrub and tree leaves, while grassland plants are often dominated by *n*-C_{31} alkanes (*Eglinton* and *Hamilton*, 1967; *Schwark* et al., 2002). Therefore, the ratio of *n*-C_{27} to *n*-C_{31} alkanes has been frequently used to calculate relative input of *n*-alkanes from different vegetation in soils and sediments including other similar molecular ratios such as *n*-C_{25+27}/*n*-C_{31+33} used in an identical way.

2.7 Statistical analysis

The data set (bulk C, N, total lipid concentration, alkane composition, CPI, ACL, and molecular ratios) was tested for significant differences using one-way analysis of variance (ANOVA) and a significance level of $P < 5\%$, followed by post hoc Scheffé test. The statistical evaluation was performed

with R studio software (*R Development Core Team*, 2010). Additionally, the student t-test (with a significance level of P < 5%, using Microsoft Excel) for paired samples was accomplished to check if individual samples showed any significant long-term effect of drought *vs*. control.

3 Results

3.1 Bulk elemental analysis: C and N concentration

The above-ground biomass of all three grassland plant species showed almost identical C concentration under control conditions (Tab. 1). The averaged C concentration of heathland above-ground biomass was significantly higher *vs*. that of grassland. The C concentration of grassland roots was higher than that of heathland. The C concentrations of grassland and heathland soil samples did not differ significantly from each other (Tab. 1). Compared to control conditions, no significant effect on the C concentration was observed one year after the last drought for the whole sample set.

The total N concentration of the above-ground biomass was significantly higher for grassland *vs*. heathland plants under control conditions (Tab. 1). The highest N concentration was observed for the legume *L. corniculatus vs*. all other plants. The N concentration of root and soil samples was lower for heathland than for grassland. Compared with the control, the N concentration of all samples did not differ significantly one year after the last drought.

All plant samples (except *L. corniculatus*) revealed high C : N values (> 20; Fig. 1A). The soil samples were characterized by lower C : N values (< 20). The values were significantly lower for grassland than heathland above-ground biomass. For soil and root samples, no differences were observed between grassland and heathland. The repeated annual drought had no significant effect on C : N values for plant and soil samples in the long-term.

3.2 Bulk isotope values (δ^{13}C and δ^{15}N)

Under control conditions, the δ^{13}C values of all above-ground biomass samples ranged between –28.0‰ and –30.6‰ (Fig. 1B). The averaged δ^{13}C value of heathland above-ground biomass was higher compared to that of grassland. Also heathland soils revealed an enrichment in ^{13}C compared to grassland soils. For above-ground biomass, only *C. vulgaris* revealed a 1‰ higher δ^{13}C value one year after repeated drought. Heathland soil exhibited ^{13}C enrichment one year after drought *vs.* control conditions, which was however not significant. No difference was observed for grassland soil and roots one year after drought *vs.* control conditions.

The averaged δ^{15}N values of above-ground biomass samples ranged between –0.3 and –5.7‰ under control conditions (Fig. 1C). *H. lanatus* (–0.3 ± 0.5‰) revealed the highest enrichment in ^{15}N in the sample set and *C. vulgaris* (–5.7 ± 0.9‰) the strongest depletion in ^{15}N. Heathland above-ground biomass exhibited lower δ^{15}N values compared to grassland above-ground biomass. The repeated

Table 1: Bulk C and N concentrations, as well as total lipid concentration normalized to C content of grassland and heathland model ecosystems under control and one year after repeated annual drought conditions (mean ± SE, *n* = 5 field replicates).[a]

Model ecosystem	Sample type	Plant species	C concentration (mg g^{-1} dry wt.)		N concentration (mg g^{-1} dry wt.)		Total lipid concentration (mg g^{-1} C)	
			Control	Drought	Control	Drought	Control	Drought
Grassland	Above-ground biomass	*P. lanceolata*	429.3 ± 2.6	427.7 ± 4.9	11.0 ± 1.0	10.9 ± 1.0	71.7 ± 8.0	72.9 ± 4.5
		H. lanatus	422.0 ± 5.5	421.3 ± 7.3	16.8 ± 0.9	11.8 ± 0.3	115.1 ± 8.7	112.1 ± 11.7
		L. corniculatus	433.6 ± 8.8	432.3 ± 1.8	33.4 ± 1.9	30.5 ± 1.9	105.2 ± 3.5	101.5 ± 11.2
		avg. above-ground biomass	428.3 ± 3.5	425.4 ± 2.8	20.3 ± 2.6	16.9 ± 3.1	97.3 ± 7.4	95.7 ± 7.5
	Roots		380.1 ± 28.5	366.1 ± 15.7	12.2 ± 0.6	11.9 ± 1.4	54.8 ± 4.6	82.9 ± 40.1
	Soil		24.4 ± 2.6	26.5 ± 1.0	1.6 ± 0.2	1.7 ± 0.1	42.9 ± 4.5	39.3 ± 3.5
Heathland	Above-ground biomass	*V. myrtillus*	472.0 ± 4.9	478.3 ± 4.2	8.5 ± 0.5	10.5 ± 0.9	134.9 ± 25.0	110.5 ± 8.3
		C. vulgaris	463.6 ± 3.1	460.0 ± 9.1	10.2 ± 0.4	10.5 ± 0.2	131.9 ± 16.8	137.5 ± 19.4
		avg. above-ground biomass	467.8 ± 3.2	469.1 ± 6.1	9.7 ± 0.5	9.2 ± 0.3	133.4 ± 13.5	123.8 ± 11.2
	Roots		348.6 ± 42.2	nd	8.5 ± 0.6	nd	82.0 ± 15.2	nd
	Soil		25.0 ± 1.4	23.6 ± 1.4	1.4 ± 0.1	1.3 ± 0.1	49.3 ± 2.1	49.1 ± 3.5

[a] nd: not detected.

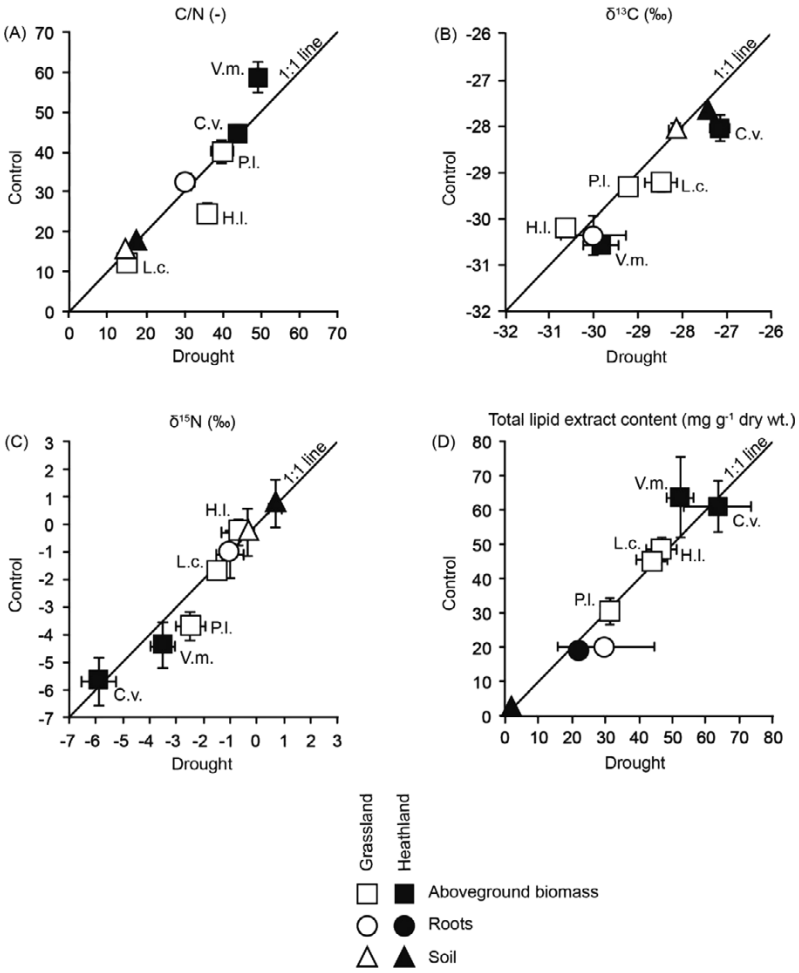

Figure 1: C : N ratio (A), stable C ($\delta^{13}C$; B), N ($\delta^{15}N$; C), isotope composition, and total lipid concentration (D) under control and one year after repeated annual drought treatment. Values (mean ± SE, n = 5) are given as means with standard errors of five field replicates. Abbreviations: V.m. (*Vaccinium myrtillus*), C.v. (*Calluna vulgaris*), P.I. (*Plantago lanceolata*), H.I. (*Holcus lanatus*), L.c. (*Lotus corniculatus*).

drought treatment did not significantly change the $\delta^{15}N$ values for all plant and soil samples in the long-term.

The annual recurrent drought was expected to result in ^{13}C enrichment in soils. Although the drought period started always in May, the period of the most active biomass production, in which increase in biomass was prohibited due to drought, it was assumed that the biomass production during the drought period corresponded to the mentioned percentages to the isotope values of the plant biomass entering the soil as no data were available for net biomass increase and organic matter incorporation into soil. For the whole growth season the ^{13}C enrichment for grassland plants was estimated to account for 0.5‰ to 0.6‰ and for heathland 0.3‰ to 0.4‰ (Tab. 2). The measured data confirmed the expected change in $\delta^{13}C$ values for heathland soil with an enrichment of 0.2‰. However, no change in $\delta^{13}C$ value for grassland soil was observed after five years of repeated annual drought.

3.3 Total lipid concentration and *n*-alkane distribution pattern

The highest total lipid extract (TLE) concentration normalized to dry wt. was observed in shoots and decreased towards roots and soil (Fig. 1D). The averaged TLE concentration was higher for heathland (62.4 ± 6.2 mg g^{-1}) *vs.* grassland above-ground biomass (41.6 ± 3.1 mg g^{-1}) under control conditions. The TLE was lower (6%) for heathland roots *vs.* grassland roots, whereas no differences were observed for soils. One year after the repeated drought, the TLE concentration was lower for *V. myrtillus* by 17% compared to control conditions, which was, however, not significant. Also, TLE concentrations of grassland and heathland roots and soils were not affected significantly one year after repeated drought treatment.

TLE normalized to total C for grassland was lower for both, above-ground biomass and roots *vs.* the respective heathland samples under control conditions. No significant difference was observed between grassland *vs.* heathland soils. One year after repeated drought *vs.* control conditions, only the TLE normalized to total C for grassland roots increased by 44% which was, however, not significant. All other samples did not show significant changes after applied drought *vs*. control.

The *n*-alkane distribution patterns of grassland above-ground biomass samples revealed a considerable predominance of long-chain odd *n*-alkanes maximizing either at C_{27}, C_{29} or C_{31}, depending on the plant species (Fig. 2). The same predominance of odd long-chain alkanes was observed for heathland above-ground biomass, but the most abundant compound was always *n*-C_{31}. The distribution pattern of *n*-alkanes for heathland root and soil samples followed a similar pattern as for shoot biomass with a lower predominance of *n*-C_{31} in soil. Grassland roots did not show a predominance of a single alkane, but they were also enriched by odd *n*-alkane homologues. The grassland soil revealed a comparable alkane pattern like grassland roots. A significant decrease in the relative abundance of *n*-C_{29} was observed for *P. lanceolata* above-ground biomass samples one year after repeated drought conditions. For all other plant and soil samples the *n*-alkane distribution pattern was not significantly different one year after repeated drought.

Table 2: Estimation of drought-induced change in $\delta^{13}C$ (expressed in ‰) in plants and its contribution in soil in the year 2005–2010 in the EVENT I experiment in Bayreuth Germany.

Model ecosystem	Year of drought	Estimated $\delta^{13}C$ change in plants under drought *vs.* control over the whole growth season	Expected change in $\delta^{13}C$ of soil (if 10% of soil C is replaced)
Grassland	2005	0.52	0.05
	2006	0.50	0.05
	2007	0.50	0.05
	2008	0.60	0.06
	2009	0.60	0.06
	2010	0.60	0.06
Total	2005–2010		0.33
Heathland	2005	0.26	0.03
	2006	0.26	0.03
	2007	0.25	0.03
	2008	0.40	0.04
	2009	0.40	0.04
	2010	0.40	0.04
Total	2005–2010		0.20

3.4 *n*-Alkane proxies

The CPI values for shoot biomass under control conditions ranged from 5.5 to 17.5 for grassland and heathland above-ground biomass (Fig. 3A). The values for roots and soils showed much less scatter than those of above-ground biomass samples and ranged from 4.6 to 5.8. The averaged CPI values of above-ground biomass did not show a difference between grassland and heathland under control conditions. Only for *L. corniculatus* above-ground biomass CPI value was higher one year after repeated drought *vs.* control, whereas all other biomass samples lacked significant differences. Further, no differences were observed between root and soil samples of grassland *vs.* heathland under control conditions and also no effect of repeated drought was observed for all other samples after the last drought.

The ACL values of the sample set ranged from 27 to 30, with the highest values for above-ground plant biomass and low values for soil and grassland root samples (Fig. 3B). Compared with control conditions, ACL values of all other plant samples were not different one year after repeated drought conditions, except for *L. corniculatus* that displayed a decreased ACL value, which was, however, not significant. No differences were observed for roots. Heathland soil revealed higher ACL value one year after repeated drought.

The $n\text{-}C_{27}/n\text{-}C_{31}$ ratio was characterized by values ranging from 0.4 to 1.3 over the whole sample set (Fig. 3C). Most of the grassland above-ground biomass, root, and soil samples revealed higher values than those of heathland under control conditions. One year after repeated drought conditions, the $n\text{-}C_{27}/n\text{-}C_{31}$ ratio higher was only for *L. corniculatus* than for control conditions, whereas no changes were observed for all other above-ground biomass samples. For grassland root samples, the ratio was higher one year after repeated drought *vs.* control conditions. No effect of drought was found for heathland and grassland soil compared to control in the long-term after the last drought. The ratio $n\text{-}C_{25+27} : n\text{-}C_{31+33}$ was calculated as a further molecular proxy to determine whether the trend of $n\text{-}C_{27} : n\text{-}C_{31}$ ratio can be generalized (Fig. 3D). Apart from minor variation, the ratio $n\text{-}C_{25+27} : n\text{-}C_{31+33}$ followed the same trend as $n\text{-}C_{27} : n\text{-}C_{31}$. The ratio was higher one year after repeated drought *vs.* control only for *L. corniculatus* and grassland root samples, whereas other samples did not show differences one year after repeated annual drought.

4 Discussion

4.1 Biogeochemical composition of grassland and heathland model ecosystems

Carbon and N concentrations as well as C : N ratios of grassland and heathland plots were in a typical range for common plants and soil in natural grassland and heathland ecosystems (*Bull* et al., 2000; *Billings*, 2006). Typically, for plant samples high N concentrations are observed for legumes (*Spehn* et al., 2002), as confirmed here for *L. corniculatus*. Likewise, the plant C and N concentrations and the C : N ratios of the soils were in a typical range for grassland and heathland soils (*Jensen* et al., 2003; *Rowe* et al., 2006). The $\delta^{13}C$ values of all above-ground biomass samples ranged between –28.0‰ and –30.5‰ (Fig. 1B), consistent with val-

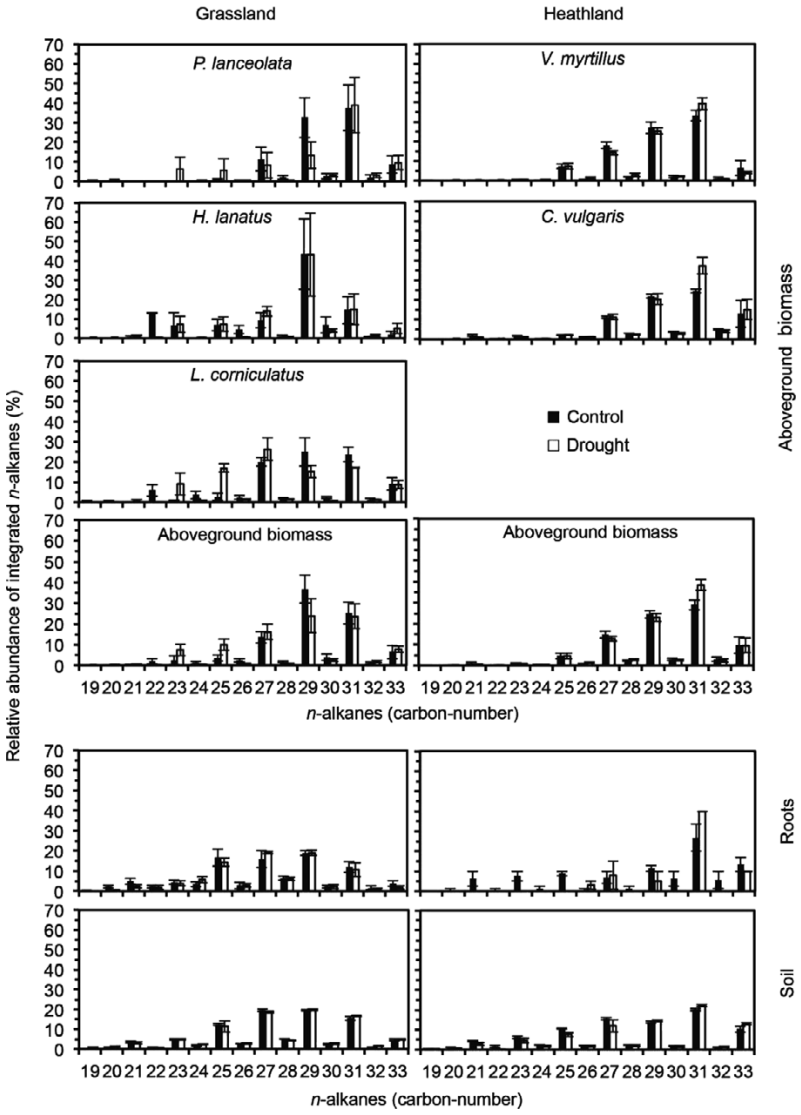

Figure 2: Relative abundance of integrated *n*-alkanes in plant and soil samples exposed to repeated annual drought and control treatment for 5 years. Values (mean ± SE, *n* = 5) are given as means with standard errors of five field replicates.

ues expected for C_3 vegetation (*Farquhar* et al., 1989). The $\delta^{13}C$ values for the individual heathland and grassland plant species confirmed literature results (*Huang* et al., 1997; *Dungait* et al., 2008). The $\delta^{15}N$ values for heathland and grassland plant species ranged between –0.3‰ and –5.7‰ as found previously (*Gerdol* et al., 2000; *Spehn* et al., 2002; Fig. 1C). All above-ground biomass showed depleted $\delta^{15}N$ values compared to $\delta^{15}N$ of bulk soil, the latter ranging between –0.4 and 0.7‰ (Fig. 1C), which confirms literature results (*Amundson* et al., 2003; *Kahmen* et al., 2008).

The $\delta^{15}N$ values of plant tissue can be influenced by multiple N sources that are commonly used by plants (*Högberg*, 1997). After incorporation of plant tissues into soil, processes in soil (*e.g.*, nitrification, denitrification, and ammonification) can affect $\delta^{15}N$ values, leading to ^{15}N enrichment with degradation of plant tissues (*Dawson* et al., 2002). In the current study, this led to slight enrichment (max. 3‰ for *P. lanceolata*) in ^{15}N in grassland soil compared to the corresponding plant biomass, whereas for heathland on average a higher enrichment (5‰) was observed.

All above-ground biomass samples in the model ecosystems investigated revealed the highest TLE concentration normalized to dry wt. for shoots and decreased towards roots and soil (Fig. 1D, Tab. 1). CPI and ACL values of *n*-alkanes were highest for shoots and decreased towards roots and soil (Fig. 3A, B), in agreement with previous observations (*Dawson* et al., 2002).

4.2 Differences between grassland and heathland model ecosystems

The highest $\delta^{13}C$ value was observed for *C. vulgaris* compared to that of other investigated heathland and grassland plants. Heathland soil also displayed higher $\delta^{13}C$ values compared to those of grassland, which indicates higher proportion of ^{13}C-enriched organic matter incorporated in heathland soil compared to that of grassland (Fig.1B). In contrast, depleted ^{15}N values were observed for above-ground biomass samples of heathland *vs.* grassland species (Fig 1C). However, although no heathland roots were available, the soil samples did not correspond with the differences observed for above-ground biomass. Plant $\delta^{15}N$ values of roots and soil reflect mineral N as a source of root N from the soil (*Högberg*, 1997). Moreover, the $\delta^{15}N$ value of soil may reflect an increasing rate of soil N cycling, indicating loss of ^{15}N-depleted mineral N and leading to gradual ^{15}N enrichment of soil (*Amundson* et al., 2003; *Kahmen* et al., 2008). The TLE concentrations were higher in heathland than grassland above-ground biomass samples, which indicate a higher proportion of compounds with a lower degradability of plant organic matter in heathland *vs.* grassland (*Marschner* et al., 2008). In roots, C and N concentrations were higher for grassland than for heathland. However, for soil, almost identical C and N concentrations were observed for heathland and grassland. Most of the differences observed for plant tissues could not be traced in soil, most likely due to the comparatively short duration of the experiment on previously homogenized soils and the minor magnitude of the differences in plant tissues.

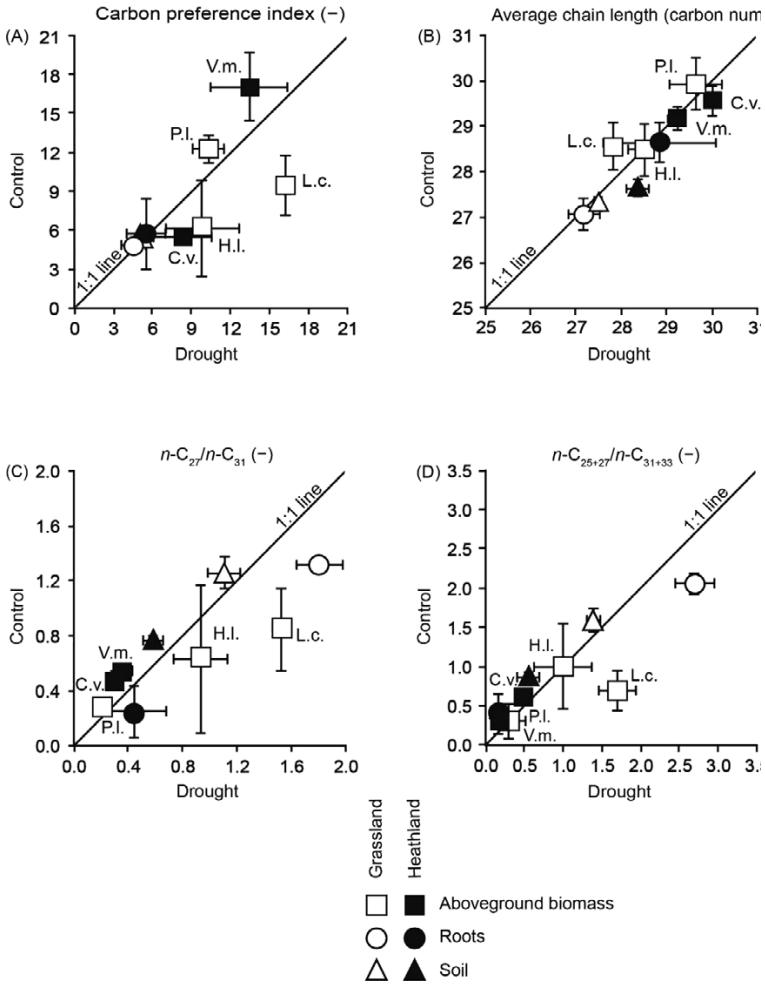

Figure 3: Carbon preference index (A), average chain length (B), *n*-alkane ratios n-C_{27} : n-C_{31} (C), n-C_{25+27} : n-C_{31+33} (D) under control and one year after repeated annual drought treatment. Values (mean ± SE, $n = 5$) are given as means with standard errors of five field replicates. Abbreviations: V.m. (*Vaccinium myrtillus*), C.v. (*Calluna vulgaris*) P.I. (*Plantago lanceolata*), H.I. (*Holcus lanatus*), L.c. (*Lotus corniculatus*).

CPI and ACL values of *n*-alkanes showed no significant differences between grassland and heathland above-ground biomass, but revealed a wide scatter among the different plant species, which is a common feature (*Harwood* and *Russell*, 1984; *Rao* et al., 2009). Nevertheless, both heathland plant species had higher ACL values than most of the other plant and soil samples, except for *P. lanceolata*. The ACL values for heathland roots and soil were higher than those of grassland, as confirmed by lower values for n-C_{27} : n-C_{31} and n-C_{25+27} : n-C_{31+33} ratios for heathland (Fig. 3). Thus, heathland plants that typically grow on well-drained sandy soils with a lower pH (*Roem* and *Berendse*, 2000), where drought seasons can occur frequently due to low water-holding capacity of the soil (*Schmidt* et al., 2004), contain comparatively larger amounts of alkanes with an extended C chain (*Salasoo*, 1989). This adaptation of the protective hydrophobic wax layer is not only reflected in plants, but was also observed in soils after five years of the experiment. However, not all above-ground tissues confirmed this tendency and general conclusions related to the difference between the plant communities are not possible based on the limited number of species included in the current study.

4.3 Effect of annual recurrent drought

According to previous drought experiments, we expected increased C : N ratios, δ^{13}C values (*Farquhar* et al., 1989), and δ^{15}N values (*Handley* et al., 1999) in shoots, roots and soil exposed to recurrent annual drought. However, our plant data did not confirm this tendency except for *C. vulgaris*. Consequently, no change was observed for grassland soil δ^{13}C values, whereas only a slight enrichment by 0.2‰ was determined for heathland soil. The observed change for the heathland soil was exactly the shift, which can be expected when taking into account biomass productivity and annual incorporation rates of plant organic matter into soil by 5–10% matter (*Wiesenberg* et al., 2008; *Dorodnikov* et al., 2011; Tab. 2). The discrepancy between expectations and observations might be explained by the fact that annual grassland plants renew their biomass rather fast by re-sprouting after drought (*Xu* et al., 2010) and therefore show no sustainable effect of drought on δ^{13}C values one year after the last drought and subsequently in soils.

According to our first hypothesis, we expected significant changes in the plant and soil lipid composition for heathland but not for grassland due to the recurrent annual drought. Surprisingly, among all plant samples we determined a significant influence of annual recurrent drought on the alkane composition including increased CPI, n-C_{27} : n-C_{31} and n-C_{25+27} : n-C_{31+33} ratios only for *L. corniculatus* shoots and grassland roots (Fig. 3A, C, D). The absence of significant changes argues for absence of a sustainable effect of drought on the plant lipid metabolism of the investigated plants, which was already described for different plant species (*Shepherd* and *Griffiths*, 2006). Although significant effects of drought were not observed for heathland plants, ACL of soil was significantly higher, when exposed to annual drought. This might indicate a reduced microbial activity one year after repeated drought, which led to a decrease of the decomposition of organic matter in soil (*Tiemann* and *Billings*, 2011; *Sanaullah* et al., 2012).

Our second hypothesis, which stated that C concentration decreased in soil exposed to recurrent annual drought, was not confirmed. Further, we expected a relative increase in

TLE compared to C concentration in plants and soil. Also this was not confirmed and, therefore, the hypothesis had to be rejected. The missing changes in C and TLE concentration are attributed to the fact that plants recovered quickly after the drought period and consequently changes in C and lipid cycling in the plant–soil system could not be monitored even after six extreme drought seasons. Taking into account C and lipid turnover times in soils in the range of several decades, longer experiments might be required to trace drought-induced changes in soils (*Marschner* et al., 2008).

5 Conclusions

The current study suggests that mainly short-term effects in terms of C and N cycling prevail even after extreme drought events. A sustained effect of several recurrent extreme drought phases was observed only for stable C isotope composition in plants and soils. Further research is required on lipid changes in plant–soil systems during long-term drought periods, where potentially more severe changes in the alkane composition might be observed. To test for general differences in lipid composition between grassland and heathland plant communities and their effect on C cycling and storage in soils, it is suggested to include a larger number of plant species.

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Kavita Srivastava (70%)	Sample collection, sample preparation for lipid analysis and bulk elemental and compound specific isotope analysis using GC-IRMS in the University of Zurich. Responsible for the execution of the data acquisition, statistical analysis of data and their illustration in tables and figures. Prepared the manuscript under the supervision of Guido L.B. Wiesenberg.
Guido L.B. Wiesenberg (30%)	Concept and idea of the experiment. Samples collected during the different phases of the drought period. Supervised sample analyses using gas chromatography coupled to an isotope ratio mass spectrometer (GC-IRMS) in the University of Zurich. Supervised the study and contributed intellectually to the data interpretation in the manuscript.

Severe drought influenced composition and $\delta^{13}C$ of plant and soil *n*-alkanes in model temperate grassland and heathland ecosystems

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Abstract

Drought events are predicted to increase under future climate change. In temperate ecosystems, plants are capable of resisting drought due to their hydrophobic wax layer, where *n*-alkanes are important constituents. In soils, plant-derived *n*-alkanes are comparatively resistant to degradation. To improve understanding of the significance of *n*-alkanes in plant-soil systems during a severe drought period (104 days), we investigated bulk organic C (C_{org}) concentration, total lipid extract (TLE) concentration, *n*-alkane molecular proxies and compound-specific isotope composition ($\delta^{13}C$) in model temperate grassland and heathland plant-soil systems. Heathland plants and soil revealed significantly higher concentration of C_{org}, and TLE compared with grassland. TLE and alkane composition responded quickly during the first drought phase (0 – 40 days). This indicates that plants actively utilized C and improved their *n*-alkane biosynthesis in order to withstand drought, which was confirmed by increased (2 – 3‰) $\delta^{13}C$ values for *n*-alkanes in shoot biomass. However, during later drought phases all the parameters remained constant for plants and soils. This suggests limited synthesis and cycling of plant lipids such as *n*-alkanes during intense drought. Surprisingly, increased average chain length and preference of odd *n*-alkanes in soil during the first drought phase demonstrated a rapid input of plant-derived long chain *n*-alkanes to soil, which was not expected due to the decadal residence time of alkanes in soil. The study enabled tracing of plant metabolic response in terms of alkane biosynthesis under different phases of drought and rapid cycling of alkanes in the plant-soil system.

Keywords

Average chain length; carbon preference index; compound specific isotope analysis; EVENT I experiment; plant-soil system

1. Introduction

Temperate grassland ecosystems play a crucial role in the global carbon (C) cycle (Reichstein et al., 2013; Lei et al., 2016), which has been facing increasing frequency of summer drought periods during the past few decades. In Central Europe, a higher frequency of drought periods is predicted to occur during the most active season (April – September) of plant growth (Lindborg, 2007; Rowell, 2009). Drought has the potential to influence C cycling in the plant-soil system (Fuchslueger, 2014). Its impact on plant C uptake, biosynthesis, incorporation and fate of plant-derived compounds in soil is only fragmentarily known. This demands improved understanding of C cycling in the plant-soil system exposed to drought (Reichstein et al., 2013). Among plant derived compounds, lipids are regarded as important plant constituents that are susceptible to drought stress (Shepherd and Griffiths, 2006). The lipid constituents of cuticular wax contain a complex mixture of long chain fatty acids, aldehydes, alkanes, alcohols and ketones (Post-Beittenmiller, 1996).

The amount of wax in plants, of which long chain *n*-alkanes are important constituents, can rapidly increase during the leaf growth phase in spring and early summer and remains constant throughout in the rest of the growing season (Tipple et al., 2013). However, other studies have revealed that the relative proportion of *n*-alkane production changes throughout the growing season (Chikaraishi et al., 2004; Chikaraishi and Naraoka, 2006), indicating that *n*-alkane production and variation depend on plant species and growth (Bush and McInerney, 2013) as well as on the environmental conditions to which the plants are exposed (Shepherd and Griffiths, 2006; Duan and He, 2011).

In theory, an increased amount of cuticular wax and specifically greater production of long chain *n*-alkanes would be expected under drought, because the hydrophobicity of *n*-alkanes is directly related to their chain length, as suggested by Shepherd and Griffiths (2006), and observed for many plants, such as rice (Islam et al., 2009), wheatgrass and oat (Jefferson et al., 1989). However, it remains unclear as to which time and stage of the plant growth and drought period the maximum concentration of *n*-alkanes can be observed and to which extent it is influenced by drought. This entails further questions, as many of the studies that describe seasonal and environmental effects such as drought on plant *n*-alkane composition analysed rather young plants aged e.g. weeks to months (Tipple et al., 2013). Hence, it remains unclear how mature grassland or heathland plants would modify their *n*-alkane composition, if they were exposed to an extended drought period. So far, most studies of drought effects on plant *n*-alkane composition did not include roots, although in the roots of selected species large

amounts of lipids such as alkanes are produced and released into the soil (Huang et al., 2011; Gamarra and Kahmen, 2015). Therefore, the extent to which degree root lipids are influenced by drought is largely unknown.

In soil, a major part of lipids originates from plant input (Mucawi 1981) through several pathways such as litter fall, deposition of abraised waxes (Conte et al., 2003), as well as roots and rhizodeposits and used as a molecular biomarker (Wiesenberg et al., 2010). Apart from plants, soil fauna and microorganism also contribute to soil lipids (Lorenz et al., 2007). Among the class of *n*-alkanes, long chain homologues (> C_{25}) mainly derive from plants, whereas alkanes with a shorter chain length can be attributed also to soil fauna and microorganisms (Bray and Evans, 1961). Long chain *n*-alkanes in soils are comparatively resistant against degradation (Schwark et al., 2002), which is indicated by slower turnover compared to bulk carbon and other organic substances (Marschner et al., 2008). However, knowledge is lacking so far on the impact of drought on *n*-alkane cycling in the plant-soil system.

In addition to alkane composition, compound specific $\delta^{13}C$ analysis of long chain *n*-alkanes has been frequently used to improve understanding of environmental impact on the biosynthesis and cycling of *n*-alkanes in the plant-soil system (Collister et al., 1994; Lockheart et al., 1997; Chikaraishi and Naraoka, 2003). It is obvious that, if drought stress causes a ^{13}C-enrichment for bulk C (Farquhar et al., 1989), biosynthesis of *n*-alkanes of plants exposed to drought should also lead to higher $\delta^{13}C$ values in the plants due to the coupling of the general photosysnthesis of plants and the biosynthetic isotope fractionation of lipids (Chikaraishi et al., 2004). However, to the best of our knowledge, studies showing the connection of increasing bulk $\delta^{13}C$ values and compound-specific $\delta^{13}C$ values of *n*-alkanes of plants exposed to drought are scarce. Furthermore, it is questionable, if and to which extent e.g. drought stress-induced higher production of alkanes might lead to change even in soil $\delta^{13}C$ values of *n*-alkanes (Wiesenberg et al., 2004) despite their slow turnover on a range of decades (Marschner et al., 2008).

To improve understanding of the impact of drought on *n*-alkane dynamics in the plant-soil system, we investigated model temperate grassland and heathland ecosystems that had been exposed to an extended, unprecedented drought for 104 days. Our study was guided by the following hypotheses: (i) Lipid concentration and *n*-alkane chain length increase, as do $\delta^{13}C$ values in shoots and roots under drought stress and subsequently to a minor degree also in soils exposed to drought; (ii) Greater changes would expected for temperate grassland than for

heathland ecosystems due to the higher persistence and slower reactivity of the heathland ecosystem compared with the grassland ecosystem.

2. Material and methods

2.1. Study site

The field study was conducted as part of the EVENT I experiment (Jentsch et al., 2007, 2011) at the Ecological-Botanical Garden of the University of Bayreuth, Germany [49°55‘19’ N, 11°34’55’’ E, 365 m above sea level (a.s.l.)]. The mean annual air temperature and annual precipitation were 8.2˚C and 724 mm, respectively. The upper soil (0 − 20 cm) was produced from homogenized topsoil from a nearby quarry distributed on homogenised sand from the same quarry (20 – 80 cm). The initial texture of the soil was loamy sand (820 g/kg sand, 130 g/kg silt, 50 g/kg clay). In the upper soil layer, the pH was 4.5 and in the lower layer 6.2.

The setup of the EVENT I experiment was based on a latin square design with a plot size of 2 × 2 m and in total 150 plots were exposed to several pre-treatments such as ambient control, drought, heavy rainfall, freeze-thaw cycles during the 2005 to 2010 (Jentsch et al., 2007). All treatments had been maintained for five replicate plots. The plant community of the grassland plots was initially established as a mixed culture with *Plantago lanceolata* (*P. lanceolata*), *Holcus lanatus* (*H. lanatus*), *Lotus corniculatus* (*L. corniculatus*) and *Arrhenatherum elatius* (*A. elatius*). For heathland, a mixed culture of *Calluna vulgaris (C. vulgaris)* and *Vaccinium myrtillus (V. myrtillus)* was chosen. We focused on only one annual grassland plant (*H. lanatus*) and one perennial heathland plant *(C. vulgaris)* for the study. From 11th to 13th May 2011, all plots received water treatment to the same soil moisture content for all plots that were pre-exposed to drought and control treatments since 2005 – 2011. From 17th May to 28th August 2011, a severe drought (unprecedented) experiment was conducted, which exceeded the projected climate change scenario and lasted for 104 days. To achieve this, a large rainout shelter was constructed on a steel frame (Haygrove Tunnels Ltd., Ledbury) covering all 150 plots of the EVENT I experiment and covered with transparent polythene foil (folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany). The foil permitted nearly 90% penetration of photosynthetically active radiation (Backhaus et al., 2014). Since the beginning of the severe drought, the volumetric soil water content (vol%) was measured weekly for each plot over the course of the experiment (Backhaus et al., 2014). The volumetric soil water content dropped below the permanent wilting point (7 vol%) after 10 days (27 May 2011) of the drought phase and remained constant until the end of the severe drought (28 August 2011)

period (Backhaus et al., 2014). The drought period of 104 days was divided here into three phases, drought phase I, II and III. Drought phase I corresponded to the initial drought period, i.e. 0 – 40 days, when plants were still healthy and green. Drought phase II represented a strong drought, which occurred between days 40 and 70, where photosynthetic and biosynthetic activity of the plants obviously became limited, indicated by wilting of plant leaves. Finally, drought phase III (70 – 100 days) represented the duration of the time when almost no further CO_2 uptake was possible and shoots looked almost dead (Srivastava et al., under revision, b).

The pre-treatments of 100 – 1000 yr. extreme drought events did not lead to any significant difference of C/N ratios, $\delta^{13}C$, $\delta^{15}N$ and *n*-alkane composition for all plant and soil samples compared with ambient control pre-treatments (Srivastava et al., under revision, a). Therefore, we used all replicate plots of drought and control pre-treatment as field replicates, leading to a total of 10 field replicates for grassland and 10 field replicates for heathland plant-soil systems.

2.2. Sample collection and preparation

Plants and soil samples were collected from the selected 20 plots from May – August 2011. The collection of shoot and soil samples was performed biweekly (such as on days 0, 12, 27, 40, 54, 68, 82, 96 and after 103 days of severe drought) from the individual plots. Samples of shoots were collected by cutting and pooling several green leaves for grassland or branches with green leaves for heathland plants and kept separately for the 10 field replicates. For collection of soil samples, an auger (length 15 cm, i.d 5 cm) was used, which was introduced 3× per plot and the aliquots were combined, keeping the 10 field replicates separate. All shoot and soil samples were oven dried at 40 °C. Roots were removed from soil samples and separated from the soil matrix with tweezers, washed with de-ionized water and oven dried at 40 °C. Soil samples were dry sieved to a particle size < 2 mm. All samples were ground in a ball mill (Retsch MM 200, Germany) to fine powder.

2.3. Bulk carbon and stable carbon ($\delta^{13}C$) isotopic analysis

Shoot, root (1 mg each) and soil (10 mg) samples were weighed in Sn capsules and analysed to determine bulk C concentration and the stable carbon ($\delta^{13}C$) isotope composition. As all samples were free of carbonate, the bulk C concentration is equivalent to organic C (C_{org}) concentration, this being relevant for the isotope values. Measurements were performed using an elemental analyser (Hekatech, Euro) coupled to a ConFlow III interface (Thermo Fisher,

Bremen, Germany). Combustion of samples was followed by gas chromatography (GC) separation and transferring sample gas to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany). Calibration was carried out using sucrose (IAEA-CH-6, IAEA, Vienna, Austria), polyethylene (IAEA-CH-7) and the soil reference material Chernozem (Etzdorf). Within a measurement sequence, all standards were measured repeatedly together with the samples. Isotope ratio values ($\delta^{13}C$) are expressed as per mil δ (‰) values relative to the Vienna-Pee Dee Belemnite (V-PDB) standard and calculated on the basis of:

$$\delta^{13}C = [(R_{(sample)}/R_{(standard)}-1)] \times 10^3 \quad (1)$$

where $R_{(sample)}$ is the ratio of the heavy isotope (^{13}C) to the respective light isotope (^{12}C) of the sample and the respective standard, with $R_{(standard)} = 0.0112372$ for V-PDB. Unfortunately, no isotope data were available for sampling days 12 and 96, because of a ^{13}C labelling experiment conducted on the sampled subplots.

2.4. Total lipid extract (TLE)

Total extractable lipids were extracted via Soxhlet extraction for shoots (*C. vulgaris* and *H. lanatus*), roots and soil samples using CH_2Cl_2/CH_3OH (93:7;v/v). Root samples represented a mixture of four grassland plants or two heathland plants, respectively. For roots and shoots 0.5 − 2.0 g were extracted and 40 − 50 g for soil. Extraction time was at least 36 h (Wiesenberg and Gocke, 2017). After evaporation of the solvent under atmospheric conditions, the TLE concentration was determined gravimetrically. Afterwards, the extract was sequentially separated into 4 fractions based on polarity. Neutral lipid and fatty acid fractions were obtained after solid phase extraction with KOH-coated silica gel (Wiesenberg and Gocke, 2017). Neutral lipids were eluted with CH_2Cl_2 followed by fatty acids, which were recovered with CH_2Cl_2/CH_2O_2 (99:1; v:v). Afterwards, lipid fractions were dried again and neutral lipids were further separated into aliphatic, aromatic and low polarity hetero compounds using a column filled with activated silica gel (100 Å). Aliphatic hydrocarbons were eluted with C_6H_{14} followed by aromatic hydrocarbons with C_6H_{14}/CH_2Cl_2 (1:1, v:v), and low polarity hetero compounds with CH_2Cl_2/CH_3OH (93:7, v:v; Wiesenberg and Gocke, 2017). Volume reduction was performed via rotary evaporation. The sequential separation yielded four lipid fractions, of which only aliphatic hydrocarbons were studied in detail in the current study as they were expected to yield the strongest drought related change.

2.5. GC

For identification and quantification, a defined amount of deuteriated *n*-alkane standard (D_{50}-*n*-C_{24}) was added to the individual aliphatic hydrocarbon fractions as internal standard before GC analysis. Compound identification was performed with a gas chromatograph (Agilent 6890N) coupled to a mass spectrometer (Agilent 5973N). Quantification was performed using GC with flame ionisation detection (GC–FID; Agilent 7890B). In both instruments, a J&W DB-5ms column (50 m x 0.200 mm; i.d. 0.33 µm film thickness) was used with He (purity 5.0) as carrier gas at 1 ml/min; 1µl sample was injected via an autosampler into a split/splitless injector. After a static phase of 2 min at 70°C, the GC oven was programmed to 320°C (held 20 min) at 5°C/min.

2.6. *n*-Alkane molecular proxies

2.6.1. Average chain length (ACL)

ACL has been used to differentiate between predominantly higher plant-derived long chain *n*-alkanes (Eglinton et al, 1962) and microorganism-derived organic matter (OM; Bray and Evans, 1961) in soils and sediments and is calculated as follows:

$$ACL = \sum(Z_n \times n)/\sum(Z_n), \quad (2)$$

where n is the number of carbons in the alkanes and Z_n the amount of alkanes with n carbons with n in the range $C_{17–33}$. High ACL values (≥ 25) derive from higher plant biomass (Kolattukudy, 1970), which is dominated by long chain *n*-alkanes (C_{25-33}; Eglinton *et al.*, 1962). In contrast, the contribution from short chain *n*-alkanes (C_{15-23}) that derives mainly from microbial biomass and degradation processes (Harwood and Russell, 1984) leads to low ACL values (< 25; Bray and Evans, 1961).

2.6.2. Carbon preference index (CPI)

Higher plant biomass typically shows a strong predominance of long chain odd homologous of *n*-alkanes (Eglinton et al, 1962; Kolattukudy, 1970), whereas even homologous mainly derive from degradation of OM (Zhou et al., 2005). Such predominance of odd vs. even homologues can be expressed by CPI:

$$CPI = [(\sum C_{23–33odd} / \sum C_{22–32\ even}) + (\sum C_{23–33\ odd} / \sum C_{24–34even})]/2 \quad (3)$$

where $\sum C_{23-33odd}$ represents the sum of the relative amounts of odd *n*-alkanes of a chain length of 23-33 carbon atoms and $\sum C_{22-32\ even}$ and $\sum C_{24-34even}$ the sum of even *n*-alkane homologues with a chain length of 22-32 and 24-34 carbon atoms, respectively. High values (> 10) are indicative for fresh leaf biomass, while values around 1 indicate strong degradation of OM and microorganism-derived OM (Cranwell, 1981).

2.7. Compound specific isotope analysis (CSIA)

The ^{13}C isotopic ratio values of individual *n*-alkanes were determined under continuous flow using a ThermoScientific Trace 1310 GC interfaced on-line via a GC-Isolink II to a ConFlow IV and Delta V Plus isotope ratio mass spectrometer. A TG-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The GC temperature program for *n*-alkane separation was 70 °C (held 2 min) to 320°C (held 20 min) at 5°C/min. He (purity 5.0) was used as carrier gas at a constant 1 ml/min. CO_2 of known $\delta^{13}C$ composition was automatically introduced via ConFlow IV into the isotopic ratio mass spectrometer in a series of 5 pulses at the beginning and 4 pulses the end of each analysis, respectively, and used as reference gas during every measurement.

Prior to C isotope analysis, the CO_2 reference gas was calibrated relative to V-PDB using A6 and B4 *n*-alkane mixtures provided by A. Schimmelmann (Indiana University, Bloomington, IN, USA) and instrument performance was routinely checked using an internal *n*-alkane standard mixture (*n*-C_{20} to *n*-C_{32}; Sigma Aldrich) with known $\delta^{13}C$ values. Calibration of these substances was done by combustion in an elemental analyser (EA Flash 2000) and measurement of the separated CO_2 in a Delta V Plus isotope ratio mass spectrometer after passing through the ConFlow IV interface (EA-IRMS). Precision for replicate measurements of the standard *n*-alkanes ranged between 0.05 and 0.08 for individual alkanes. Isotopic values are reported as $\delta^{13}C$ values relative to V-PDB, averaging at least three replicate measurements. The averaged values of the CSIA results were used for calculation of the weighted average isotope composition of the five most abundant compounds (*n*-C_{25}, *n*-C_{27}, *n*-C_{29}, *n*-C_{31}, *n*-C_{33}) and normalized to the proportion of each compound:

$$\text{Weighted average of } n\text{-alkane (‰)} = (A \times \delta_A) + (B \times \delta_B) + (C \times \delta_C) + (D \times \delta_D) + (E \times \delta_E)/\sum(A{:}E) \quad (4)$$

where A, B, C, D and E represent the relative proportion of the most abundant compounds and δ_A, δ_B, δ_C, δ_D and δ_E their $\delta^{13}C$ isotopic values.

2.8. Statistical analysis

The bulk C_{org}, TLE, CPI, ACL molecular proxies were tested for significant differences between grassland and heathland model ecosystems. Additionally, significant differences between the control (samples collected on day 0) and during the drought period were determined using Student's t-test for paired samples with a significance level of $P < 0.05$. The statistical evaluation was performed with R studio software (R development core team 2014).

3. Results and discussion

3.1. C_{org} concentration and TLE concentration

In general, the C_{org} and TLE concentration were higher for shoots followed by roots and soil for grassland as well as for heathland ecosystems (Table 1). TLE concentration ranged between 9 – 16% C_{org} for shoots and decreased towards roots (4 – 9%) and soil (2 – 5%). Such patterns are a typical feature of plant lipids resulting from a large amount of plant internal and cuticular waxes (Kolattukudy 1976; Wiesenberg et al., 2012).

C_{org} for *H. lanatus* (grassland plant) and *C. vulgaris* (heathland plant) showed almost identical concentration on day 0, in agreement with previous studies (Huang et al., 1997; Poorter and De Jong, 1999). TLE concentration and TLE normalized to C_{org} were significantly lower (p = 0.01) for *H. lanatus* vs. *C. vulgaris*. Higher TLE for *C. vulgaris* is associated with the typical features of perennial heathland plants, which often have thicker epicuticular wax layers as they usually grow under conditions with low water availability, where they use their wax layer for protection against water loss (Salasoo, 1987).

According to our first hypothesis above, we expected to observe increasing TLE concentration when plants were exposed to drought. However, contrary to our expectation, TLE concentration decreased (p = 0.05) by 28% for *C. vulgaris* and 10% for *H. lanatus* (not significant; p = 0.4) within 12 days of drought compared with the initial sampling on day 0. This is likely related to the modification of lipid biosynthesis under drought (Laribi et al., 2009). Interestingly, during the end of drought phase I, a significant increase (p = 0.01) in TLE concentration was observed for *H. lanatus* (61.0 ± 7.0 mg/g dry wt) until day 40, which was the maximum TLE concentration of the whole observation period. TLE concentration for *H. lanatus* decreased (21%; p = 0.05) again after 40 days and returned to the pre-drought level on day 54 (48.8 ± 2.4 mg/g dry wt) and did not change significantly during the remaining observation period. After 54 days of drought, TLE of *C. vulgaris* revealed the maximum value

(82.0 ± 4.0 mg/g dry wt) for the observation period, which was significantly higher (p = 0.01) than the value on day 0. Afterwards, TLE did not change significantly for the rest of the drought period. The changes of the TLE concentration especially during the first two drought phases (until day 54) indicate that, during the ongoing drought period, biosynthesis of plant constituents changes to increasing amount of hydrophobic components such as lipids related to overall biomass as an acclimatization to drought conditions (Hamrouni et al., 2001; González and Ayerbe, 2010).

During drought phases II and III, significantly lower C_{org} and TLE concentration, as well as TLE normalized to C_{org} (p = 0.04), were observed for grassland shoots vs. heathland shoots, which is in line with the expectations of our second hypothesis. Since the current study is the first to investigate the lipid composition in temperate grassland and heathland shoots during a severe drought of 104 days, the observed changes in the TLE could not be verified by other studies.

The grassland roots represented a mixture of four species and the heathland roots a mixture of two plant species. During sampling, it could be observed that the fibrous root systems of the grass species were closely connected to each other and produced a densely rooted depth interval in the upper 5 - 10 cm of the soil. For heathland, the roots occurred much more locally around the individual stems of the plant individuals, which is why in some soil cores only a very low amount of root biomass could be retrieved. C_{org} did not show any difference between grassland and heathland roots during drought phase I. For roots from both ecosystems, C_{org} increased significantly (p = 0.05) during drought phase II and later decreased during drought phase III. However, during drought phases II and III grassland roots revealed significantly lower (p = 0.05) C_{org} than that of heathland except for day 82.

The differences between grassland and heathland C_{org} concentration might be related to the thicker, woody tap-root tissues of heathland plants vs. the more fibrous roots of grassland species, entailing a different chemical composition. TLE concentration and TLE normalized to C_{org} were significantly lower (p = 0.01) for grassland roots vs. heathland roots at the first sampling date. TLE of heathland roots decreased by 35% (p = 0.05) after 12 days of drought, whereas no change was observed for grassland roots for the same interval. Maximum TLE was observed for grassland roots (25.5 ± 3.5 mg/g dry wt) on 40 days of drought. During drought phases II and III, TLE concentration did not considerably change in roots for both communities. In general, less variability in TLE of roots compared with shoot biomass argues for much less drought-stress related effects on biosynthesis within roots vs. shoots. This might

be related to the suberin vs. cutin biopolymer structure of root vs. shoot tissues (Kolattukudy, 1981).

The C_{org} concentration for the grassland and heathland soils is consistent with the literature (de Brogniez et al., 2015). No significant difference was observed for C_{org} between grassland and heathland soils. C_{org} of grassland and heathland soils decreased (29%; $p = 0.01$) within the first 12 days of the observation period. However, during the end of drought phase I, an increase in C_{org} was observed (34%, $p = 0.01$) for both ecosystems. Afterwards, C_{org} did not show significantly different values during drought phases II and III in comparison with day 0. The significantly different value of C_{org} for only one sampling (day 12) is quite surprising and uncommon in soils, as soil C pools typically do not significantly change within a few weeks. As soil lipid composition also in part revealed differences for this specific sampling date (see below) vs. other sampling dates, it is thought that, during sample collection or sample preparation an analytical error occurred for samples for this specific day, which could not be identified. Therefore, we regard this specific sampling point with caution, but try to focus more on general trends that could be observed over several weeks.

TLE concentration and TLE normalized to C_{org} were significantly lower ($p = 0.01$) for grassland soil than heathland soil during the whole period except for day 12. For the latter, no significant difference was observed. Similarly, as for grassland shoots and heathland shoot as well as in root samples, TLE decreased ($p = 0.01$) for grassland (17%) and heathland (35%) soils within the first 12 days. The grassland soil did not reveal any change in TLE concentration during drought phases II and III. For heathland soil TLE increased significantly ($p = 0.01$) on 40 days when compared with the control on day 0. Afterwards, no significant changes were observed for grassland soil and heathland soil drought phases II and III. In general, shifts in soil TLE concentration during the first drought phase and the absence of changes thereafter is in line with changes observed for shoot and root TLE. Further explanations of shifts in TLE are discussed below from the alkane composition.

Table 1. Bulk C_{org}, TLE and TLE normalized to C_{org} in grassland and heathland ecosystems exposed to drought. Mean ± standard errors of mean are given (n = 10). Day '0' represents control.

Ecosystem type	Sample type	Drought phase	Sampling time (days)	C_{org} concentration (mg/g dry wt.)	TLE concentration (mg/g dry wt.)	TLE /C_{org} (mg/g dry wt.)
Grassland	*H. lanatus*	I	0	512.7 ± 27.2	49.0 ± 1.7	98.5 ± 6.1
			12	499.3 ± 37.9	44.4 ± 4.0	88.9 ± 4.0
			27	495.3 ± 20.8	49.1 ± 4.1	101.8 ± 10.9
		II	40	469.9 ± 19.1	61.0 ± 7.0	124.7 ± 15.1
			54	493.3 ± 22.5	48.8 ± 2.4	99.9 ± 5.1
			68	428.4 ± 32.6	40.5 ± 1.7	101.3 ± 10.8
		III	82	468.4 ± 27.9	39.6 ± 2.3	89.3 ± 11.3
			96	498.6 ± 32.9	47.9 ± 5.4	96.6 ± 9.6
			103	435.6 ± 25.3	46.9 ± 5.8	115.7 ± 18.4
	Roots	I	0	383.8 ± 19.7	18.2 ± 1.4	48.7 ± 4.5
			12	392.2 ± 26.9	21.2 ± 1.1	55.3 ± 5.8
			27	401.7 ± 5.0	21.5 ± 1.3	53.3 ± 2.8
		II	40	430.8 ± 9.9	25.5 ± 3.5	59.1 ± 8.0
			54	542.9 ± 24.2	25.3 ± 2.4	48.7 ± 6.4
			68	386.7 ± 16.3	24.3 ± 2.6	62.1 ± 5.3
		III	82	420.6 ± 21.9	23.9 ± 4.3	56.9 ± 10.1
			96	403.6 ± 52.3	24.3 ± 1.9	63.5 ± 6.0
			103	346.9 ± 35.5	23.0 ± 3.3	67.2 ± 7.7
	Soil	I	0	25.8 ± 1.5	1.1 ± 0.1	40.9 ± 3.5
			12	18.7 ± 1.9	0.9 ± 0.0	48.3 ± 4.0
			27	30.9 ± 1.7	0.9 ± 0.0	28.9 ± 2.1
		II	40	34.7 ± 1.9	1.0 ± 0.1	26.9 ± 2.1
			54	34.4 ± 2.7	1.0 ± 0.0	32.4 ± 2.6
			68	31.3 ± 2.7	0.9 ± 0.0	31.8 ± 3.2
		III	82	32.1 ± 2.7	0.9 ± 0.0	29.8 ± 3.5
			96	33.3 ± 1.7	0.8 ± 0.1	21.5 ± 2.6
			103	32.3 ± 2.6	0.7 ± 0.0	23.5 ± 2.3
Heathland	*C. vulgaris*	I	0	527.8 ± 6.5	68.6 ± 3.3	130.2 ± 6.5
			12	499.7 ± 31.3	49.3 ± 10.3	102.6 ± 21.3
			27	537.9 ± 45.8	65.3 ± 11.0	120.9 ± 19.8
		II	40	493.7 ± 42.1	67.6 ± 3.8	146.3 ± 16.9
			54	540.1 ± 31.2	82.0 ± 4.0	168.5 ± 24.4
			68	523.4 ± 37.0	70.0 ± 4.9	136.9 ± 9.4

		III	82	564.5 ± 37.5	67.6 ± 5.9	123.2 ± 12.2
			96	648.4 ± 15.9	77.2 ± 7.6	118.8 ± 11.1
			103	591.3 ± 50.8	77.8 ± 4.4	142.9 ± 17.2
	Roots	I	0	315.0 ± 0.0	32.1 ± 3.4	94.1 ± 0.0
			12	414.7 ± 20.8	20.8 ± 1.6	50.1 ± 2.1
			27	460.9 ± 20.1	22.2 ± 2.8	48.8 ± 6.1
		II	40	514.3 ± 09.7	25.7 ± 1.2	50.1 ± 2.4
			54	564.8 ± 18.9	24.7 ± 1.8	43.6 ± 2.5
			68	469.0 ± 15.2	23.1 ± 1.5	49.6 ± 3.4
		III	82	334.5 ± 29.5	21.6 ± 2.2	64.4 ± 4.9
			96	485.9 ± 16.8	20.8 ± 3.0	43.2 ± 6.8
			103	464.8 ± 25.2	20.7 ± 1.6	40.9 ± 3.8
	Soils	I	0	24.3 ± 1.3	1.2 ± 0.1	49.4 ± 2.5
			12	17.4 ± 2.1	0.8 ± 0.1	45.0 ± 2.1
			27	35.1 ± 2.1	1.2 ± 0.1	34.9 ± 2.4
		II	40	36.8 ± 3.9	1.7 ± 0.1	47.6 ± 5.4
			54	36.6 ± 2.6	1.5 ± 0.1	44.3 ± 5.2
			68	35.4 ± 3.8	1.4 ± 0.1	42.7 ± 3.8
		III	82	28.8 ± 1.4	1.0 ± 0.1	34.1 ± 2.7
			96	30.8 ± 1.8	0.8 ± 0.1	26.1 ± 1.8
			103	28.7 ± 3.5	0.8 ± 0.0	26.6 ± 2.2

TLE, Total lipid extract

3.2. *n*-Alkane molecular proxies

3.2.1. CPI and ACL

CPI and ACL of *n*-alkanes have been frequently applied proxies for source apportionment of shoot- and root-derived *n*-alkanes (Gocke et al., 2013). In general, the CPI for grassland shoot biomass is characterized by significantly (p = 0.01) higher values than corresponding root biomass (Fig. 1), confirming previous literature (Huang et al., 2011). This is related to the strong predominance of long chain *n*-alkanes (*n*-C_{29}, *n*-C_{31} *n*-C_{33}) in leaves, whereas roots have a lower predominance of odd long chain *n*-alkanes (Huang et al., 2011; Angst et al., 2016). ACL did not differ significantly between shoot and root biomass for grassland. The CPI values of grassland soil samples ranged between 5 and 8, which were intermediate between shoot and root biomass of the respective ecosystems. For the grassland ecosystem, soil, root and shoot samples revealed identical ACL values. Thus, ACL and CPI values of the grassland ecosystem suggest that the source of the soil *n*-alkanes samples is a mixture of root and shoot biomass.

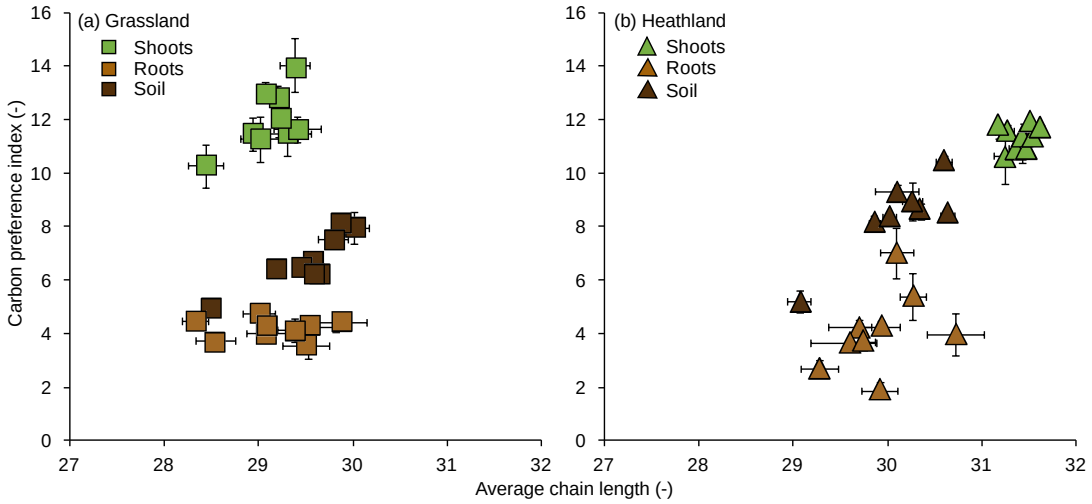

Figure 1. Carbon preference index (CPI) vs. average chain length (ACL) of long-chain *n*-alkanes (C_{25} – C_{33}) showing the source discrimination and apportionment in shoots, roots and soil of (a) grassland and (b) heathland ecosystems. Mean ± standard errors of the mean are given (n = 10 field replicates).

Heathland shoots showed significantly higher CPI and ACL (p = 0.01) values for shoots than roots, confirming previous literature (Huang et al., 2011; Angst et al., 2016). The differences between the *n*-alkane composition of root and shoot biomass is related to the different

biochemical structure of both plant tissues, with cuticular wax *n*-alkanes being more significant for leaf than for root biomass (Kolattukudy, 1981).

The CPI and ACL values for heathland soil ranged from 5 – 10 and 29 – 30.5, respectively. The CPI and ACL values for soil samples can be directly related to the living plant biomass degradation or selective preservation of very long chain *n*-alkanes (Buggle et al., 2010; Lichtfouse et al. 1998). The ACL values of heathland soil and roots were almost identical, but lower than shoot biomass. In general, the magnitude of ACL and CPI values are in line with previous studies, applying these ratios for source apportionment (Gocke et al., 2013). Lower ACL values of soil than shoot biomass might indicate that not *C. vulgaris* but the other plant species on the plots (*V. myrtillus*) might contribute to a greater extent to shoot-derived *n*-alkanes in soil.

Overall, the ACL values of all sample types like shoot and root biomass as well as soil of the grassland ecosystem were significantly lower ($p = 0.001$) vs. the corresponding sample types of the heathland ecosystem. For CPI, no differences were observed between the grassland and heathland plant-soil systems. The difference in ACL is in line with heathland ecosystems and grassland ecosystems (Diefendorf and Freimuth, 2017, Jansen and Wiesenberg, 2017).

It was expected to observe an increase in long chain *n*-alkanes concentration in shoot biomass, once they were exposed to a drought period of several weeks (Shepherd and Griffiths, 2006; Kosma et al., 2009), subsequently raising ACL values. Furthermore, an increase in the production of odd long chain *n*-alkanes was also expected to increase the CPI. However, after 12 days of drought a significant decrease ($p = 0.04$) in ACL and CPI values was observed for *H. lanatus* compared with day 0. Afterwards, no significant change in ACL and CPI values was observed for grassland shoots during the remaining observation period. The decrease in ACL and CPI during drought phase I could be a result of a specific adaptation of the investigated grassland plant, which was already observed for other plants (Zhang et al., 2005; Duan and He, 2011).

The ACL and CPI values did not change during the drought period for the heathland plant *C. vulgaris* (Supplementary information, Table S1). Such a lack of change could be attributed to the fact that no adaptation in the biosynthesis of longer chain *n*-alkanes occurred during the drought. This supports the fact that heathland plants with thick leaves and woody tissues are well adapted in terms of their alkane biosynthesis to the often water limited conditions in

heathlands, which is different from grassland plants that grow under various moisture regimes (Kirkels et al. 2013; Diefendorf and Freimuth, 2017; Jansen and Wiesenberg, 2017).

ACL of grassland roots increased (p = 0.05) during the first 12 days of drought (Supplementary information, Table S1) and remained almost constant during later drought phases with the exception of day 54, where a significantly higher value (p = 0.01) was observed vs. adjacent sampling dates. No significant shift was observed for CPI values of grassland roots during the whole observation period.

Opposite to heathland shoots, CPI of heathland roots decreased with observation time (Supplementary information, Table S1). ACL for heathland roots decreased (p = 0.01) until day 40 of the drought phase and remained almost constant until the end of the drought. The decrease in ACL and CPI in roots during drought might indicate an increased production rate of alkanes, where chain elongation is reduced and the production of byproducts (Post-Beittenmiller 1996; Shepherd and Griffiths, 2006) such as even alkane homologues leading to lowering of CPI. This overall trend suggested that production of long chain *n*-alkanes is a key response of plants against drought (Kosma et al., 2009; Guo et al., 2015).

ACL and CPI significantly increased (p = 0.05) for grassland and heathland soils during the first 12 days of drought and remained almost constant until the end of the drought period (Supplementary information, Table S1). The quick response of soil ACL and CPI values was surprising, as significant changes could not be expected in soil, because of the large alkane pool in soil that is characterized by a decadal turnover time (Marschner et al. 2008). However, likely due to plant exposure to drought stress, an increased release of cuticular alkanes as abraded waxes as well as root-derived alkanes and a subsequent incorporation of these plant-derived alkanes into soil seem to be responsible for the observed change. During later drought phases, no significant change was observed for soil *n*-alkanes. This is not surprising if we take into account that during drought phases II and III, plant C uptake and translocation towards soil decreased significantly compared with drought phase I (Srivastava et al., under revision). Furthermore, the direct input of plant-derived *n*-alkanes during the drought phase seemed to equilibrate degradation of soil alkanes, thereby leading to almost constant alkane composition during the remaining drought season after day 12 (Eglinton and Eglinton, 2008; Buggle et al., 2010).

3.2.2. Relative abundance of long chain *n*-alkanes

To obtain more specific information for source apportionment, the relative composition of most abundant plant-derived long chain *n*-alkanes (*n*–C_{25} to C_{35}) were examined (Fig. 2 and Supplementary information, Table S1). The relative proportion of the most abundant long chain *n*-alkanes has been frequently used for source apportionment of soil *n*-alkanes (Schwark et al. 2002; Huang et al., 2011; Buggle et al., 2010).

H. lanatus was characterized by an equal relative proportion (39.2 ± 3.0%) of *n*-C_{25+27}, and *n*-C_{31+33} (38.1 ± 5.4%) at day 0, which has also been already described, elsewhere (Bush and McInerney, 2013; Kirkels et al., 2013). *n*-C_{25+27} were the most abundant long chain *n*-alkanes of grassland roots (48.2 ± 2.3%) and soil (43.4 ± 1.0%), which showed the maximum at day 0.

Heathland shoots revealed a high proportion of *n*-C_{31+33} (85.0 ± 0.5) vs. other *n*-alkanes on day 0. Like heathland shoots, roots (58.9 ± 5.7) and soil (40.4 ± 4.7) revealed comparatively higher proportions of *n*-C_{31+33} than *n*-C_{29} and *n*-$C_{25+27.}$ A strong enrichment of *n*-C_{31+33} in heathland shoots, roots and soil is in agreement with the literature (Huang et al., 1997; Kirkels et al. 2013).

Within drought phase I (days 12 – 40), *H. lanatus* shoots showed an equal predominance (37.5 ± 3.2%) of *n*-C_{29} and *n*-C_{31+33} (37.6 ± 5.7%; Fig. 2 and Supplementary information Table S1). During later drought phases II and III, *n*-C_{29} became the most abundant *n*-alkane in heathland shoot biomass. The observed changes in *n*-alkane composition are likely related to the modified biosynthesis of *n*-alkanes during drought (Kim et al., 2007; Kosma et al., 2009). Heathland shoots revealed a high relative abundance (80 – 85%) of long chain *n*-C_{31+33} vs. other *n*-alkanes, with no significant trend during drought exposure.

n-C_{25+27} were the most abundant long chain *n*-alkanes of grassland roots (48.2 ± 2.3%) and soil (43.4 ± 1.0%), which showed a maximum on day 0. The abundance of *n*-C_{25+27} ranged between 30 and 50% and between 25 and 50% for *n*-C_{31+33} for grassland roots during different phases of the drought (Fig. 2 and Supplementary information Table S1). As for heathland shoots, roots also showed a relative enrichment of *n*-C_{31+33} (40 – 66%) throughout the whole observation period. Hence, heathland and grassland roots differed in their composition of long chain *n*-alkanes.

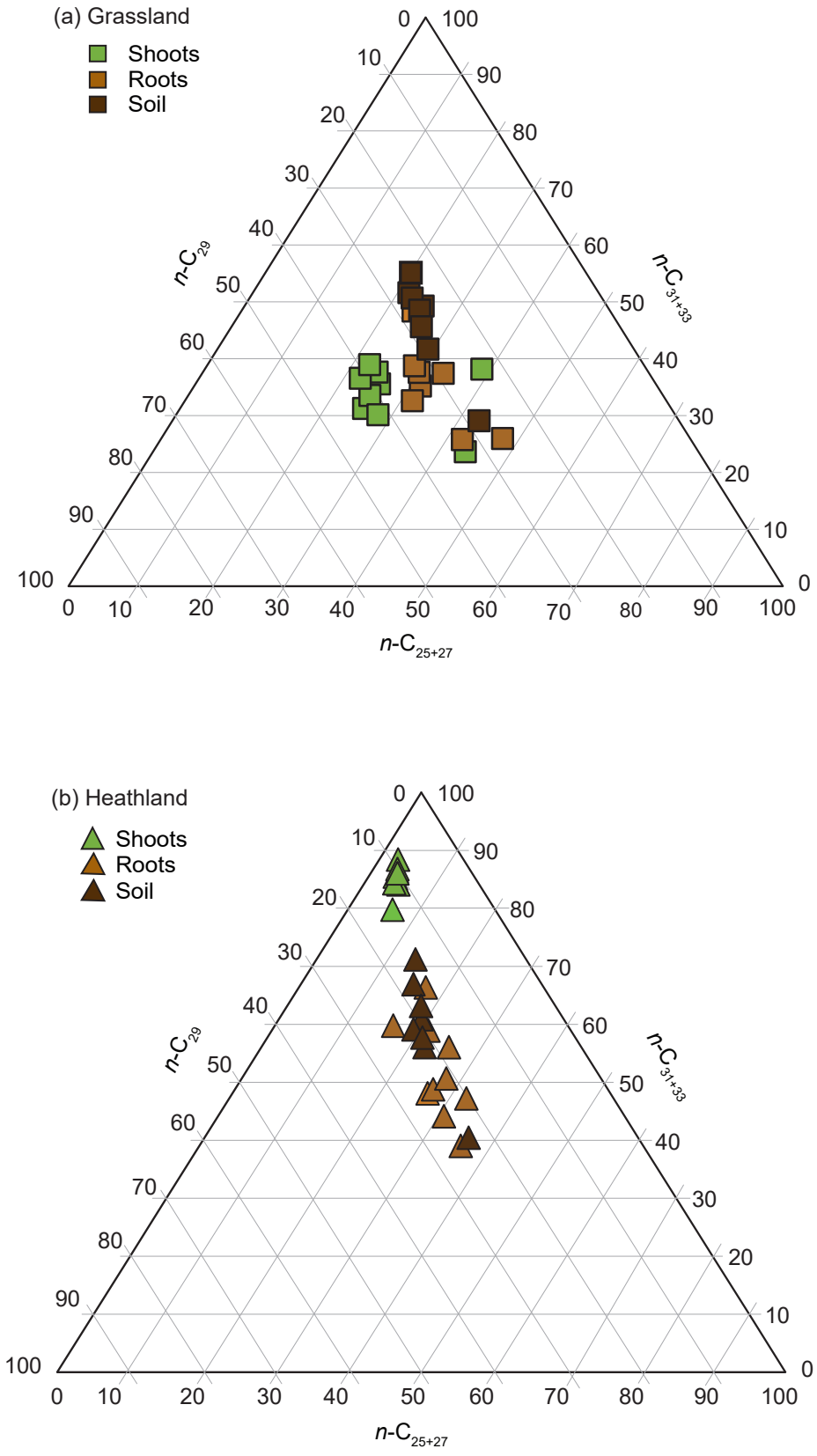

Figure 2. The relative composition of most abundant *n*-alkanes (*n*-C_{25+27}, C_{29} and C_{31+33}) in shoots, roots and soil (n = 10) of (a) grassland and (b) heathland ecosystems.

The strong enrichment of *n*-C_{25+27} in grassland soil on day 0 changed towards a predominance to *n*-C_{31+33} (40 – 55%) during drought period (Fig. 2 and Supplementary information Table S1). This reflects a direct incorporation of plant-derived long chain *n*-alkanes in soil. The predominance of *n*-C_{31} in grassland soil during the drought period, which is dissimilar to *n*-C_{29} enrichment in *H. lanatus* shoot biomass, is most likely due to a contribution of alkanes from the three other plant species that occurred on the plots, of which the alkane composition of which could not be determined due to limited resources.

A clear difference was observed between grassland (dominated by *n*-C_{29}) and heathland (dominated by *n*-C_{31}) ecosystems, in agreement with previous studies (Huang et al., 1997; Van Bergen et al., 1998). Soil alkane composition of long chain homologues was almost intermediate between above-ground and below-ground plant biomass, arguing for a mixed contribution of root- and shoot-derived OM deriving from the investigated plants. However, the other plant species that contribute in lower proportion to overall plant biomass on the plots (Backhaus et al., 2014) also contribute to soil alkane composition. This led to deviating alkane composition of soil vs. shoot OM, which is also reflected in the CPI and ACL values.

3.3. Stable isotope composition ($\delta^{13}C$)

Bulk $\delta^{13}C$ of grassland and heathland plant-soil systems varied between –25.8‰ and –30.7‰ and was in a typical range for C_3 plant-soil systems (Fig. 3 and Table 2; Huang et al., 1997; Farquhar et al., 1989). In general, grassland shoot biomass was characterized by higher $\delta^{13}C$ values than root biomass. For soil, $\delta^{13}C$ values were slightly higher than for plants throughout the observation period. As for grassland, heathland shoot biomass revealed higher $\delta^{13}C$ values vs. root biomass, where soil revealed $\delta^{13}C$ values comparable with shoot biomass. Overall, $\delta^{13}C$ of heathland plant-soil systems were slightly higher than grassland plant soil systems. Such a similarity for soil $\delta^{13}C$ values and to plant $\delta^{13}C$ values is common as soils act as integrators of the incorporated plant- and microorganism-derived OM (Wiesenberg et al., 2004, Jandl et al., 2006) of the respective ecosystems. However, slight differences for soil samples vs. plant samples could be related to the fact that in the current study not all the potential plant species that contributing to the soil OM could be analysed. On the other hand, seasonal changes might play a minor role for bulk C_{org} due to the comparatively short duration of the experiment of 6 years, resulting in considerable higher portions of bulk OM deriving from previous cultivation vs. the experiment-derived C_{org} taking into account the decadal turnover of soil OM (Marschner et al., 2008).

The compound specific $\delta^{13}C$ values of *n*-C_{25-35} alkanes for all sample types in the grassland were virtually identical on day 0 (-35.9 ± 0.2 for shoots, -34.2 ± 0.5 for roots, -35.8 ± 0.3 for soil). For heathland, root and soil alkanes revealed an identical isotope composition, whereas shoot $\delta^{13}C$ values were considerably higher throughout the observation period compared with root biomass and soil OM. In general and in accord with bulk $\delta^{13}C$ values, $\delta^{13}C$ values of alkanes were higher for the samples from heathland, than for samples from the grassland ecosystem, in agreement with previous observations (Huang et al., 1997; Chikaraishi and Naraoka, 2006; Krull et al., 2006).

Compound specific $\delta^{13}C$ values of *n*-alkanes of shoot and root biomass and soil from control samples (day 0) were depleted by ca. 3 – 7‰ relative to the bulk C_{org} for grassland and heathland ecosystem (Fig. 2 and Table 2). A similar range of differences between bulk and compound specific $\delta^{13}C$ value has been described for leaf biomass (Collister et al., 1994; Conte et al., 2003; Eley et al., 2016), roots (Wiesenberg et al., 2004; Chikaraishi and Naraoka, 2006) and soil (Cayet and Lichtfouse, 2001, Wiesenberg et al., 2004).

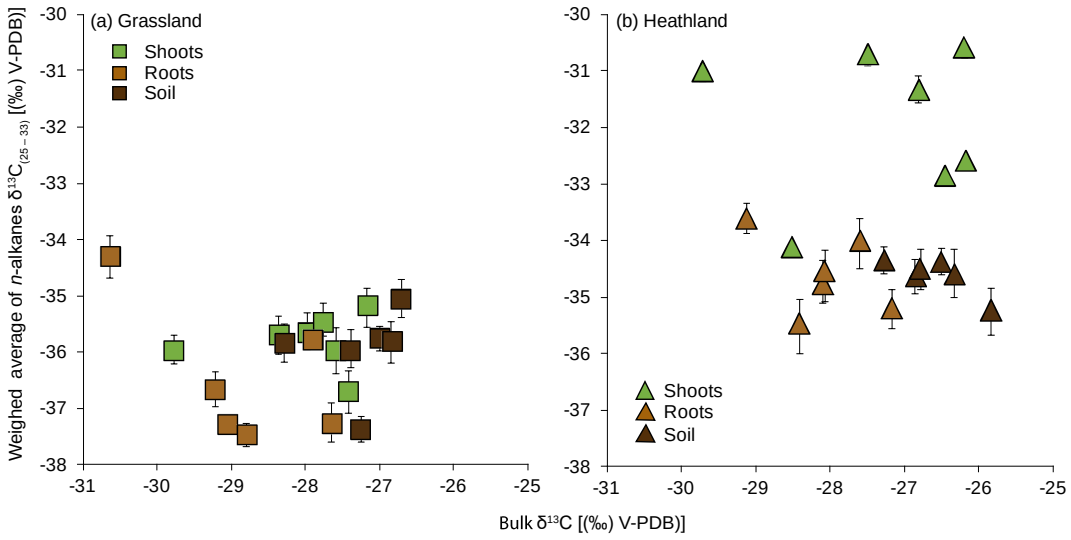

Figure 3. Weighted average of compound-specific $\delta^{13}C$ of long-chain *n*-alkanes (C_{25} – C_{33}) vs. bulk $\delta^{13}C$ isotopic composition in shoots, roots and soil of (a) grassland and (b) heathland ecosystems.

An increase in bulk $\delta^{13}C$ values of plant tissues when exposed to drought was expected due to the reduced stomatal conductance and consequently selective higher ^{13}C values within the tissues (Farquhar et al., 1989). As the close connection of the overall biosynthesis in plant tissues and the specific *n*-alkane proxies, an increase in the compound-specific *n*-alkane $\delta^{13}C$ values was also expected (Shepherd & Griffiths, 2006). As further expected, the bulk $\delta^{13}C$

Table 2. Stable carbon isotopic compositions of *n*-alkanes and bulk C (‰ vs. V-PDB) in model grassland and heathland ecosystems. Mean ± standard errors of mean are given (measurement replicates, n = 3). Day '0' represents control.

Ecosystem type	Sample type	Drought phase	Sampling time	*n*-C_{25}	*n*-C_{27}	*n*-C_{29}	*n*-C_{31}	*n*-C_{33}	Weighted average of *n*-C_{25-33}	Bulk C
			(day)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)
Grassland	*H. lanatus*	I	0	-34.1 ± 0.1	-35.8 ± 0.2	-36.5 ± 0.3	-36.3 ± 0.6	-36.1 ± 0.4	-35.9 ± 0.2	-29.8 ± 0.0
			27	-32.2 ± 0.3	-34.3 ± 0.2	-35.9 ± 0.2	-36.6 ± 0.1	-37.2 ± 0.1	-35.2 ± 0.3	-27.2 ± 0.0
		II	40	-33.0 ± 0.0	-35.6 ± 0.3	-37.1 ± 0.2	-37.9 ± 0.2	-37.2 ± 0.8	-36.7 ± 0.4	-27.4 ± 0.0
			54	-32.1 ± 0.0	-34.2 ± 0.0	-36.1 ± 0.0	-37.5 ± 0.0	-39.1 ± 0.1	-36.0 ± 0.6	-27.6 ± 0.0
			68	-33.4 ± 0.2	-35.2 ± 0.5	-36.4 ± 0.4	-35.8 ± 0.7	-32.9 ± 0.3	-35. 6 ± 0.3	-27.9 ± 0.0
		III	82	-33.4 ± 0.4	-34.4 ± 0.1	-35.8 ± 0.2	-36.1 ± 0.2	-35.8 ± 0.1	-35.4 ± 0.3	-27.8 ± 0.0
			103	-33.9 ± 0.6	-34.5 ± 0.1	-35.9 ± 0.1	-36.5 ± 0.2	-36.9 ± 0.4	-35.7 ± 0.3	-28.4 ± 0.0
	Roots	I	0	-30.3 ± 0.0	-34.3 ± 0.1	-32.4 ± 0.1	-34.9 ± 0.1	-36.1 ± 0.1	-34.2 ± 0.5	-30.7 ± 0.0
			27	-34.5 ± 0.0	-36.3 ± 0.0	-37.3 ± 0.1	-37.6 ± 0.1	-38.2 ± 0.0	-37.2 ± 0.3	-27.6 ± 0.0
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-35.2 ± 0.4	-37.5 ± 0.5	-36.4 ± 0.4	-36.8 ± 0.8	-36.3 ± 0.4	-36.6 ± 0.3	-29.2 ± 0.0
			68	-35.4 ± 0.2	-36.1 ± 0.1	-35.8 ± 0.0	-35.7 ± 0.3	-35.5 ± 0.0	-35.7 ± 0.1	-27.8 ± 0.0
		III	82	-36.8 ± 0.2	-37.4 ± 0.3	-37.1 ± 0.1	-37.3 ± 0.1	-37.6 ± 0.2	-37.2 ± 0.1	-29.0 ± 0.0
			103	-37.2 ± 0.5	-37.8 ± 0.5	-37.5 ± 0.5	-37.4 ± 0.3	-37.0 ± 0.4	-37.4 ± 0.2	-28.8 ± 0.0
	Soils	I	0	-34.0 ± 0.2	-35.8 ± 0.1	-35.7 ± 0.1	-36.8 ± 0.1	-37.7 ± 0.1	-35.8 ± 0.3	-28.3 ± 0.0
			27	-33.2 ± 0.4	-33.4 ± 0.2	-34.7 ± 0.1	-35.5 ± 0.0	-36.4 ± 0.1	-35.0 ± 0.3	-26.7 ± 0.0
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-34.6 ± 0.5	-34.7 ± 0.5	-35.3 ± 0.3	-36.3 ± 0.2	-37.6 ± 0.4	-35.8 ± 0.4	-26.8 ± 0.0
			68	-35.3 ± 0.5	-35.7 ± 0.8	-35.6 ± 0.2	-35.7 ± 0.1	-36.1 ± 0.4	-35.7 ± 0.2	-27.0 ± 0.0
		III	82	-35.2 ± 1.0	-34.8 ± 0.6	-35.7 ± 0.6	-36.6 ± 0.3	-37.6 ± 0.2	-35.9 ± 0.3	-27.4 ± 0.0
			103	-37.4 ± 0.4	-37.8 ± 0.4	-37.6 ± 0.4	-37.3 ± 0.3	-36.3 ± 0.6	-37.3 ± 0.2	-27.3 ± 0.0
Heathland	*C. vulgaris*	I	0	-32.8 ± 0.0	-33.0 ± 0.0	-34.7 ± 0.0	-34.0 ± 0.0	-34.3 ± 0.0	-34.1 ± 0.2	-28.5 ± 0.0
			27	-32.1 ± 0.2	-32.0 ± 0.1	-32.7 ± 0.1	-32.7 ± 0.1	-32.6 ± 0.1	-32.6 ± 0.1	-26.2 ± 0.0
		II	40	-31.1 ± 0.2	-30.7 ± 0.1	-31.1 ± 0.0	-30.8 ± 0.0	-30.8 ± 0.0	-30.8 ± 0.1	-27.5 ± 0.0
			54	-30.6 ± 0.1	-30.9 ± 0.5	-31.5 ± 0.6	-31.5 ± 0.5	-31.5 ± 0.6	-31.3 ± 0.2	-26.8 ± 0.0
			68	-32.5 ± 0.2	-32.4 ± 0.2	-33.1 ± 0.1	-33.0 ± 0.5	-32.9 ± 0.1	-32.9 ± 0.1	-26.5 ± 0.0
		III	82	-30.0 ± 0.0	-30.3 ± 0.1	-30.8 ± 0.0	-30.7 ± 0.1	-30.8 ± 0.0	-30.7 ± 0.0	-26.2 ± 0.0
			103	-31.8 ± 0.4	-31.3 ± 0.3	-31.4 ± 0.1	-30.9 ± 0.1	-31.0 ± 01	-31.0 ± 0.1	-29.7 ± 0.0

	Roots	I	0	-32.6 ± 0.4	-32.6 ± 0.2	-34.2 ± 0.2	-34.3 ± 0.4	-32.9 ± 0.6	-33.6 ± 0.2	-29.2 ± 0.0
			27	-36.4 ± 0.3	-36.5 ± 0.3	-34.7 ± 0.3	-34.0 ± 0.2	-33.2 ± 0.3	-34.7 ± 0.4	-28.2 ± 0.0
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-34.2 ± 0.2	-34.6 ± 0.0	-35.0 ± 0.1	-35.3 ± 0.0	-37.0 ± 0.1	-35.6 ± 0.3	-27.2 ± 0.0
			68	-32.2 ± 0.5	-33.0 ± 0.2	-37.8 ± 0.3	-34.0 ± 0.0	-34.5 ± 0.4	-34.0 ± 0.5	-27.6 ± 0.0
		III	82	n.a.	-32.1 ± 0.0	-36.4 ± 1.5	-36.1 ± 0.1	-36.8 ± 0.5	-35.5 ± 0.6	-28.4 ± 0.0
			103	-33.8 ± 0.3	-33.2 ± 0.0	-37.9 ± 0.2	-34.3 ± 0.0	-34.2 ± 0.2	-34.6 ± 0.4	-28.1 ± 0.0
	Soils	I	0	-32.84 ±	-33.7 ± 0.1	-34.6 ± 0.0	-35.0 ± 0.1	-35.1 ± 0.0	-34.3 ± 0.2	-27.3 ± 0.0
			27	-32.28 ±	-32.9 ± 0.0	-34.8 ± 0.0	-35.7 ± 0.1	-36.2 ± 0.0	-35.2 ± 0.4	-25.8 ± 0.0
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-32.5 ± 0.1	-32.8 ± 0.0	-34.3 ± 0.2	-35.0 ± 0.2	-35.2 ± 0.2	-34.6 ± 0.3	-26.8 ± 0.0
			68	-32.7 ± 0.0	-32.9 ± 0.1	-34.4 ± 0.1	-34.3 ± 0.0	-34.9 ± 0.0	-34.3 ± 0.2	-26.5 ± 0.0
		III	82	-32.1 ± 0.1	-32.6 ± 0.1	-34.4 ± 0.1	-35.7 ± 0.0	-35.7 ± 0.3	-34.5 ± 0.4	-26.3 ± 0.0
			103	-33.1 ± 0.3	-32.7 ± 0.1	-34.4 ± 0.1	-35.4 ± 0.2	-36.1 ± 0.2	-34.5 ± 0.3	-26.8 ± 0.0

n.a., not available

values as well as compound specific $\delta^{13}C$ *n*-alkanes values for *H. lanatus* showed a 1.5‰ enrichment in ^{13}C after 27 days of the drought period, which suggested that *de-novo* synthesis of long chain *n*-alkanes during the early drought period. No significant changes were observed for *H. lanatus* after 40 days. For *C. vulgaris*, a 3‰ enrichment in ^{13}C was observed after 40 days of the drought, which remained rather constant until the end of the drought experiment. Compound-specific data for plant *n*-alkanes from similar drought experiments were not available far comparison with these data.

In contrast to shoot biomass, the compound-specific $\delta^{13}C$ values of *n*-alkanes of root biomass revealed lower values by 3‰ and 1‰ for grassland and heathland roots, respectively, after 27 days of drought. This is just the opposite trend for bulk $\delta^{13}C$ values and might be related to different biosynthesis in root vs. shoot biomass. As the same was observed for root samples of both ecosystems it is rather likely that either isotope fractionation occurs in precursor compounds of root alkanes during their formation or translocation from shoot to root biomass. During drought phases II and III, the $\delta^{13}C$ values remained almost constant for C_{org} and also for *n*-alkanes until the end of the observation period. Although opposite trends have been observed for $\delta^{13}C$ of *n*-alkanes between roots and shoot biomass, both trends are caused by the drought stress of the plants.

Similar to plant biomass, we expected to observe higher $\delta^{13}C$ values for *n*-alkanes and bulk C in soil during the drought phase, but at a lower magnitude compared with plant samples. Bulk $\delta^{13}C$ for grassland and heathland soil was 1.5‰ higher, compared with initial sampling dates during the drought phase I (27 days after drought). But the compound-specific $\delta^{13}C$ values of *n*-alkanes were 1.1‰ lower for heathland whereas 0.8‰ higher values have been taken into account for grassland soil after 27 days of drought. During the later drought phases II and III these $\delta^{13}C$ values remained almost constant in the plant-soil systems. Especially during initial drought, lower $\delta^{13}C$ values of heathland soil were in good agreement with root isotope development during the same period, but opposite to the above-ground biomass. The simultaneous evolvement of heathland soil and root alkane isotope values might indicate that, during drought phase I, a stronger input of root-derived alkanes occurred. As during later stages of the drought (phases II and III), neither changed for compound-specific $\delta^{13}C$ values of root or for soil *n*-alkanes for grassland and heathland soils and isotope values were almost identical for both sample types, further conclusions on incorporation of root-derived alkanes could not be drawn. However, greater similarities between root and soil alkanes rather than between shoot and soil alkanes argue for a predominant root-derived source of soil alkanes

here. This has been controversially discussed in the recent past, where some authors stated that roots do not play an important role in soil profiles (Schäfer et al., 2016), while others found clear evidence for the significance of root C at a molecular level (Jansen et al., 2006; Gocke et al., 2014) as well as for bulk C (Schmidt et al., 2011). Our results during initial drought support the latter, demonstrating incorporation of root-derived *n*-alkanes during the initial drought.

Overall, the increase in compound-specific $\delta^{13}C$ of *n*-alkanes during drought phase I suggested that grassland and heathland shoots were actively synthesizing long chain *n*-alkanes in order to withstand drought, supporting our first hypothesis. We observed differences especially between root alkane $\delta^{13}C$ values of heathland, which were different from grassland. Although this is not a clear evidence for our second hypothesis, we could conclude that the investigated grassland and heathland plants obviously have different strategies for adopting their lipid biosynthesis to drought.

4. Conclusions

This is the first study describing the C and lipid composition in model temperate grassland and heathland ecosystems exposed to severe drought periods lasting 104 days. It provided evidence for an active response of lipid biosynthesis to drought stress as reflected in the TLE and *n*-alkane composition of root and shoot biomass in both plant-soil systems. The strongest changes were observed for the first drought phase, lasting until day 40, while no further significant changes in lipid composition could be determined during the following drought phases II and III. Furthermore, comparison of grassland and heathland plant-soil systems highlighted differences in the response of alkane compound-specific $\delta^{13}C$ values after drought exposure, especially in their root systems, which supports different adaptation of both plant-soil systems to drought stress. Finally, the changes in soil alkane composition, especially during the first 40 days of drought argue for a subsequent incorporation, especially of root-derived alkanes under drought, which has not been observed, before. Further investigation of the effect of drought periods on lipid metabolism in plant-soil systems are required to draw general conclusions, especially due to the fact that only model temperate grassland and heathland ecosystems with several plant species were investigated here. However, the first evidence of fast adaptation of lipid cycling in the plant-soil system to initial drought highlights the significance of rhizosphere processes, even for compound classes in soil with a slow turnover, such as alkanes.

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Manuscript III

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Authors	Contributions
Kavita Srivastava (50%)	Sample collection, sample preparation for the bulk elemental and isotopic analysis (EA-IRMS). Responsible for the execution of the data acquisition, statistical analysis of data and their illustration in tables and figures. The manuscript is written together with the contribution of all co-authors.
Anke Jentsch (3%)	Main initiator of the Event experiment, where samples were collected.
Juergen Kreyling (7%)	Provided shoots and roots biomass data that were collected biweekly. Contributed in the improvement of the manuscript.
Bruno Glaser (15%)	Conducted bulk elemental (C, N) and stable isotopic (δ^{13}C) analysis (EA-IRMS) of shoots, roots and soil samples. Provided insightful feedback on the first draft of the manuscript.
Guido L.B. Wiesenberg (25%)	Concept and idea of the labelling experiment during the different phases of a severe drought. Samples collected during the different phases of the drought period. Supervised the study and contributed in the data interpretation in the manuscript.

Short-term carbon dynamics in a temperate grassland and heathland ecosystem exposed to 104 days of drought followed by irrigation

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Abstract

Temperate ecosystems are susceptible to drought events. The effect of a severe drought (104 days) followed by irrigation on the plant C uptake, its assimilation and input of C in soil were examined using a triple $^{13}CO_2$ pulse–chase labelling experiment in model grassland and heathland ecosystems. First $^{13}CO_2$ pulse at day 0 of the experiment revealed much higher ^{13}C tracer uptake for shoots, roots and soil compared to the second pulse (day 44), where all plants showed significantly lower ^{13}C tracer uptake. After the third $^{13}CO_2$ pulse (day 70), very low ^{13}C uptake in shoots led to negligible allocation of ^{13}C into roots and soil. During irrigation after the severe drought, ^{13}C tracer that was allocated in plant tissues during the second and third pulse labelling was re-allocated in roots and soil, as soon as the irrigation started. This re-allocation was higher and longer lasting in heathland compared to grassland ecosystems.

Keywords: $^{13}CO_2$ pulse labelling, severe drought, carbon tracer uptake, plant-soil system

1. Introduction

Drought events are expected to occur more frequently in Central Europe during the summer season due to climate change and increased climatic variability [1]. The functions of terrestrial ecosystem of Central and Mediterranean Europe is expected to be influenced by the frequent occurrence of drought periods [2]. In fact, these sporadic extreme events apparently might become a critical issue for predicting the change in the (C) cycle in the terrestrial ecosystems [3,4]. In particular, discerning how plant community attributes and extreme climate patterns influence rate of C uptake by plants and its input in soil is important to understand under current and future climate extremes [4]. In general, the temperate grassland ecosystem may act as C sink and can contribute to C sequestration in soil [5,6]. However, this may be jeopardized by increased frequency of drought events. Drought events potentially lead to a disturbance in the terrestrial C cycle in the plant-soil system [2,4], whereas re-irrigation is observed to re-activate C cycling the plant-soil system after drought events by regeneration of plants [7,8]. To improve our understanding with respect to the impact of climate change, it is crucial to gain more information about the influence of long-term severe drought (i.e. extended period of abnormally low precipitation) followed by re-irrigation on the C assimilation, allocation in plants and stabilization in temperate grassland and heathland soils [9,10].

Uptake and allocation of C within plants strongly depends on the demand in different plant tissues [11,12]. During the initial drought phase plants usually close their stomata to prevent water loss, which results in less CO_2 uptake and subsequently lower C assimilation [13,14]. At the interface between plant and soil, roots play a crucial role to maintain water regulation within the whole plant [15]. Therefore, relatively large amounts of C can be allocated from shoots towards roots in order to promote root growth for mining the soil for water and withstand initial drought phases [11,14]. In face of a prolonged drought, C allocation towards roots gets further limited due to reduced plant growth and root integrity [16]. Such drought-induced changes in roots may affect C assimilation and input in soil [14,17]. There are two major sources of C input in soil, i.e aboveground biomass and root biomass including litter and exudates [15,18]. Under drought, soil moisture becomes limited, especially if it drops below the permanent wilting point, which leads to the reduction of soil microbial activity [17,19]. Overall, reduced soil moisture affects litter decomposition and mineralization rate [17] and consequently C input in soil [20]. As not all published results point to the same direction, it remains controversial, how C dynamics change in soils when exposed to severe drought, i.e. either by lowering plant productivity, modified root growth or due to reduced microbial activity in soil [21]. The assessment of soil C dynamics under severe drought is important to investigate due to numerous factors influencing the involved processes [21]. In addition, nitrogen (N) availability and its dynamics also gets affected under drought due to reduced soil water and

thus lower nutrient availability in soil [22,23] which leads to change in plant growth, litter decomposition and subsequently an increase in the C:N ratio in plants [24,25] and in soil [17,26].

Apart from drought phases, irrigation after drought periods can induce a rapid response in the plant growth [7,8] but it is not clear if and to which extent the plant-soil system would recover after a severe drought period, i.e. >100 days. The drought-induced response of above- and belowground biogeochemical processes is important to understand, how C cycling in the plant-soil system is influenced by drought [27]. Furthermore, the recovery of ecosystems and C cycling after extended drought periods needs to be investigated. To conclude, it is important to improve our knowledge with respect to the response of severe drought and re-wetting on the C dynamics in the plant-soil system.

Frequently, short-term C dynamics in the plant-soil system have been investigated using $^{13}CO_2$ pulse-chase labelling experiments [28–30]. Commonly, the existing drought studies [30–32] typically did not account for extended time-series analysis of drought conditions for more than 10 weeks, followed by re-irrigation. The current study focused on a severe drought (104 days) and re-irrigation experiment that was conducted in summer 2011 in Bayreuth, Germany [33]. We conducted three $^{13}CO_2$ pulse labelling experiment during the different phases of drought to compare C uptake assimilation and allocation in the plant-soil system in a model temperate grassland and heathland ecosystem. Our study followed the hypotheses:

I. Plant C uptake and C transfer towards soil decrease with increase in the duration of the experimental drought in the year 2011.

II. Irrigation after a long-term drought in the plant-soil system can promote re-allocation of C stored in shoots towards roots and re-intensification of C cycling in the plant-soil system.

III. Heath community is more resistant against drought than grassland, whereas within communities, plants are characterized by distinct drought resistance, and thus C uptake and translocation towards soil.

2. Material and methods

2.1. ***Site description***

The experiment was established at the Ecological-Botanical Garden of the University of Bayreuth, Bayreuth, Germany [49°55‘19’ N, 11°34’55’’ E, 365 m above sea level (a.s.l.)] in the year 2005 and

named as EVENT I experiment [34]. The mean annual air temperature at the site is 8.2 °C and mean annual precipitation 724 mm. The upper soil layer (0 − 20 cm) was produced from homogenized topsoil from a nearby quarry distributed over homogenized sand from the same quarry on the lower soil layer (20 – 80 cm), where drainage was installed at the bottom. The initial texture of the soil was loamy sand (820 g kg^{-1} sand, 130 g kg^{-1} silt, 50 g kg^{-1} clay). In the upper soil layer, pH was 4.5 and in the lower layer 6.2. At the experimental site, temperate grassland and heathland plant communities of various diversity levels were initiated. Different environmental manipulations like drought, heavy rainfall, and freeze-thaw cycle could be compared with control plots that were maintained with long-term average rainfall intensities. For all different treatments and plant communities, five independent replicate plots were available. The setup of the experiment was based on a latin square design and consists of a total of 150 plots with a size 2 × 2 m for each plot [34] of which in total 20 plots have been used in the current study. In May 2011, a severe drought experiment was initiated on this experimental site. Before that, all plants received a watering treatment to adjust all plots to the same initial conditions from 11-13th May 2011. The severe drought simulation started on 17 May 2011 and was maintained until 28 August 2011 (104 days). To achieve this, the whole experimental site was covered with a large rainout shelter constructed with a steel frame (Haygrove Tunnels Ltd., Ledbury, UK) and transparent plastic sheet (0.18 mm, UV M42, folitec Agrarfolien – Vertriebs GmbH, Westerburg, Germany), which permitted nearly 90% penetration of photosynthetically active radiation [35]. After 104 days of drought, irrigation was performed using a portable irrigation system [36].

For the current study, we selected 10 plots for grassland and 10 plots for heathland plant (dwarf shrub) communities. These 20 plots included pre-treated control and drought plots (with five replicates for each treatment and community), the latter being exposed to 100 to 1000 yr. extreme [33] drought during the period 2005 – 2010. As no significant differences between differently pre-treated plots (control and drought) were observed in terms C, N concentrations analyzed in shoots, roots and soil samples collected from each plot at the beginning of the experiment, we used both pre-treated plots as independent field replicates for the current study [37]. To support the absence of differences between different pre-treatments, we tested them throughout the current experiment for significant differences in terms of C and N concentrations, C uptake and C allocation, but did not determine significant differences between pre-treated control and drought plots. Thus, in total ten replicate plots were available for grassland and heathland plant communities, respectively. Since the applied severe drought covered all plots of the EVENT I experiment, no real control plots were available throughout the drought period. Hence, we used the initial data plots at day ‘0’ as control results for the whole drought period. The plant community of the grassland plots was initially established as a mixed culture of *Plantago lanceolata* (*P. lanceolata*), *Holcus lanatus* (*H. lanatus*), *Lotus corniculatus* (*L. corniculatus*) and *Arrhenatherum elatius* (*A. elatius*). We

focused only on two grassland species, which was *P. lanceolata* and *H. lanatus* as the other plants were not available for destructive sampling for all plots and for all samplings throughout the whole experimental period. In the heathland, a mixed culture of *Calluna vulgaris (C. vulgaris)* and *Vaccinium myrtillus* (*V. myrtillus*) was chosen.

Since the beginning of the severe drought, the volumetric soil water content (vol%) measured on top-soil (10 cm soil depth) and other controlled parameters were weekly measured in each plot over the course of the experiment [35]. The volumetric soil water content dropped below the permanent wilting point (7 vol%) after 10 days (27 May 2011) of the severe drought and remained constant until the end of the severe drought (28 August 2011) period [35]. In order to determine the limit until which plant C uptake and its allocation in root biomass and soil get reduced under drought, we divided the complete drought period of 104 days into three phases, i.e. phases I, II and II (Fig 1). Phase I of the drought period was represented by days 0 – 40, which is similar to the expected 100 yr. extreme at the experimental site [34]. During phase II plants were thought to still show some resistance and maintain C cycling, which was represented by days 40 until 70. Phase III was the final phase of the severe drought (days 70 – 100), when C cycling in the plant-soil system was assumed to be completely ceased. The severe drought was followed by irrigation, to investigate the time required for re-allocation of the C stored within the shoots towards roots and soil after regeneration of the grassland and heathland ecosystems.

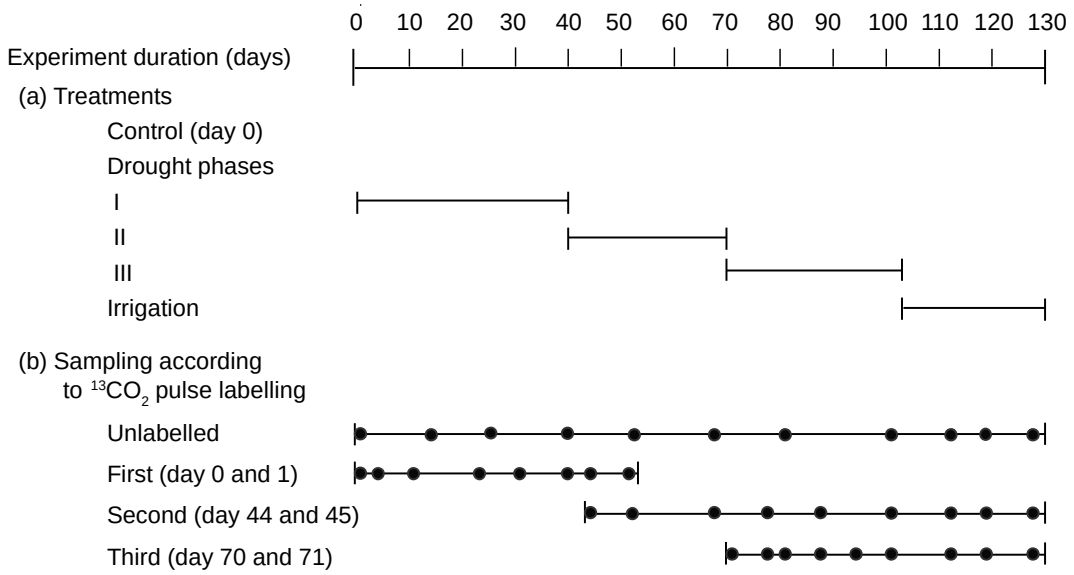

Figure 1. Experimental scheme showing (a) three different phases of drought treatment followed by irrigation and (b) sampling performed on unlabelled and labelled (after each $^{13}CO_2$ pulse labelling) plots. Dots represent the day of sampling.

2.2. $^{13}CO_2$ pulse-chase labelling

To investigate the plant C uptake and allocation of C during the different phases of a severe drought (104 days), we applied three individual $^{13}CO_2$ pulses at the drought phase I, II and II respectively. The first pulse labelling was performed on 18/19 May 2011, followed by the second (30 June/1 July 2011) and third labelling (26/27 July 2011). For each individual $^{13}CO_2$ pulse-chase experiment, one quarter (1 x 1m) of the individual plots was used, accounting for three quarters used for the subsequent labelling and keeping one quarter for ongoing long-term experiments. Six out of ten replicate plots of the individual plant communities were used for the labelling experiments and four kept as control without application of $^{13}CO_2$ pulses. The first $^{13}CO_2$ pulse experiment was carried out on six grassland plots on the first day of the drought period (May 18, 2011), starting at 09:30 CET and lasted for ca. five hours to ensure complete assimilation of labelled $^{13}CO_2$. On the next day (19 May 2011), six heathland plots were labelled with $^{13}CO_2$ for a similar duration. The labelling was conducted on a clear sunny day (ca. 25 °C at noon) to ensure active uptake of CO_2 by plants. Similar weather conditions (25 ± 2 °C, sunny days) were chosen for subsequent labelling experiments to ensure similar CO_2 uptake by plants. Each plot was labelled by placing a closed transparent labelling chamber consisting of transparent PVC (polyvinyl chloride) plastic sheets (permitted nearly 90% penetration of photosynthetically active radiation) on the plots. The aluminium base frame of the labelling chamber (1 x 1 m, 10 cm height) was inserted ca. 5 cm into the soil one day before the labelling to minimize diffusion from the labelling chambers. At the aluminium base frame a channel was filled with water after closing of the chambers to make the chambers airtight at the connection of the base frame and the plastic sheets. The height of the labelling chambers was 50 cm for grassland and 100 cm for heathland plots, respectively, due to different plant heights of the plant communities. The $^{13}CO_2$ pulse labelling experiment was achieved by hanging an open glass bottle inside the chamber ca. 20 cm above the soil surface, which was filled with $NaH^{13}CO_3$ (99.9 atom% ^{13}C) that was completely dissolved in de-ionized water. $^{13}CO_2$ was released from the labelling solution by carefully injecting 10 ml of 10% H_2SO_4 directly into the glass bottle containing the labelling solution via a syringe passing through the plastic foil. The homogeneous distribution of $^{13}CO_2$ and circulation of air inside the chamber were ensured by continuously running a battery driven fan inside the labelling chambers. After ca. five hours aluminium frames and plastic foils of the labelling chambers were removed. Following the same procedure of the first pulse labelling experiment, the second and third $^{13}CO_2$ pulse labelling experiments were conducted under almost identical conditions, first for grassland and on the day thereafter for heathland plots.

2.3. Sample collection and preparation

The first sampling was performed one day after the first $^{13}CO_2$ labelling for labelled and unlabelled plots (Fig. 1 and supplementary information S1). Subsequently, shoot samples were collected weekly and soil as well as root samples biweekly from labelled and unlabelled plots. After second and third pulse labelling, all sample types (shoot and root biomass as well as soil) have been collected weekly, starting one day after the isotopic pulse. Depending on the individual labelling experiments, samples were collected in the respective quarters of the individual plots. In each quarter, sampling was performed at least 10 cm away from any margin in order to avoid edge effects. Shoot samples of each plant species were collected by cutting them off with scissors to receive at least 10-15 green leaves or ca. 1 g dry weight by taking and pooling several green leaves for grassland plants or branches with green leaves for heathland plants. During later stages of the experiment, green leaves and branches became limited, why also partially senescent leaves and branches were collected. As aboveground biomass of some species became limited during the drought experiment, we restricted sample analysis to *P. lanceolata* and *H. lanatus* for grassland plants and analysed both heathland species. To collect soil samples, an auger (15 cm length x 5 cm inner diameter) was used, which was introduced three times per plot and the soil and root material was combined, thereafter. Immediately after sampling, all shoot and soil samples were oven dried at 40 °C. Root samples could not be collected for individual species and were kept as grassland and heathland root mixtures, respectively, after they were removed from soil samples. Root samples were retrieved by manual picking with tweezers, washing with de-ionized water and oven drying at 40 °C. Oven dried soil samples were dry sieved to a particle size < 2 mm. All samples were ground in a ball mill (Retsch MM 200, Germany) to fine powder.

2.4. Bulk elemental (C and N) and stable isotope ($\delta^{13}C$) analysis

To determine C and N contents as well as stable carbon isotope ($\delta^{13}C$) composition, soil (10 mg), shoot and root samples (1 mg each) were weighed in Sn capsules. Measurements were performed using an elemental analyzer (Hekatech, Euro) coupled to a ConFlow III interface (Thermo Fisher, Bremen, Germany). Combustion of samples was followed by gas chromatographic (GC) separation and transferring sample gas to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany). Calibration was carried out using the following standards: sucrose (IAEA-CH-6, IAEA, Vienna, Austria), polyethylene (IAEA-CH-7), glutamatic acid (USG-41, IAEA) and soil reference material, Chernozem (Etzdorf). Within a measurement sequence, selected standards were measured repeatedly together with the samples. Isotope ratio values ($\delta^{13}C$) are expressed as per mil δ (‰) values relative to the Vienna Pee Dee Belemnite (V-PDB) standard and calculated based on the following equation:

$$\delta\,^{13}\mathrm{C}\,(‰) = \left(\frac{R_{(sample)}}{R_{(standard)}} - 1\right) \times 10^3 \qquad \text{(Equation 1)}$$

Where R = $^{13}C/^{12}C$ for both, samples and standard with $R_{(standard)}$ = 0.0111802 [38,39].

In order to express the amount of ^{13}C added by pulse labelling, atom percentages in the different plant and soil compartments were calculated [30]:

$$^{13}\mathrm{C\ atom\ \%} = \frac{100 \times 0.0111802 \times \left(\frac{\delta_{(sample)}}{1000} + 1\right)}{1 + 0.0111802 \times \left(\frac{\delta}{1000} + 1\right)} \qquad \text{(Equation 2)}$$

Where $\delta_{(sample)}$ represents the isotopic values of labelled or unlabelled shoot, root and soil samples.

For all shoot, root and soil samples, the excess ^{13}C was calculated for grassland and heathland ecosystems, taking into account dry biomass pools in plant and soil compartments [30]:

$$\text{Excess } ^{13}\mathrm{C}_{sample} = \frac{^{13}\text{C atom \%}_{(\text{labelled sample})} - {}^{13}\text{C atom \%}_{(\text{unlabelled sample})}}{100} \times \text{dry weight}_{(sample)} \times \frac{\text{C\%}_{(sample)}}{100}$$

(Equation 3)

Excess ^{13}C (expressed as g m^{-2}) is the total amount of ^{13}C added by each pulse-labelling campaign to the dry weight of the plant and soil compartments and the respective C pools of the same sample compartment per ground area (each plot had a area of 1 m^2). ^{13}C atom%$_{(labelled\ sample)}$ is the atom% of the ^{13}C-labelled samples, ^{13}C atom%$_{(unlabelled\ sample)}$ is the atom% of unlabelled samples and C%$_{(sample)}$ is the C concentration (%) of the respective samples.

The relative allocation of ^{13}C in roots was calculated in relation to the initial ^{13}C excess obtained in shoot and in root biomass:

$$^{13}\mathrm{C}_{\text{relative allocation in root vs.shoot biomass}} = \frac{\text{excess } ^{13}\mathrm{C}_{\text{root biomass}}}{\text{excess } ^{13}\mathrm{C}_{\text{shoot biomass(t0)}} + \text{excess } ^{13}\mathrm{C}_{\text{root biomass}}}$$

Equation 4

Where excess $^{13}C_{shoot\ biomass(t0)}$ is the average initial ^{13}C excess in shoots determined 24 hours after each labelling campaign. However, the relative allocation of C from shoots towards roots is only calculated based on shoot biomass estimation, since the two out of four grassland plant species have not been investigated in our study. Furthermore, for heathland plants an overestimation of shoot biomass of *C.*

vulgaris led to substantial underestimation of relative ^{13}C allocation in heathland root biomass and soil organic matter.

2.5. Statistical analysis

Due to the unavailability of control samples in parallel to the drought study for 104 days, the first samples collected from all plots in May 2011 were considered as control samples for shoots, roots and soil for each parameter analysed. The data set for C:N ratios, natural variation in the bulk $\delta^{13}C$ and ^{13}C tracer excess in shoots, roots and soil were tested to examine the drought induced effect using samples collected on day 0 (considered as control) and compared to the samples collected during the drought treatment period (until 104 days). Furthermore, the C:N ratios and bulk $\delta^{13}C$ were tested for significant differences between grassland and heathland communities. The test was performed using one-way analysis of variance (ANOVA) and a significance level of $p < 0.05$, followed by post hoc Scheffe´ test. The ^{13}C excess in shoots, roots and soil was statistically tested for $^{13}CO_2$-labelled vs. unlabelled plots and also between grassland and heathland (n=6) using Student's t-test with a significance level of $p < 0.05$. The statistical evaluation was performed with R studio software [40].

3. Results

3.1. C:N ratio

For grassland shoot biomass the C:N ratio ranged between 18 and 40, whereas it varied from 25 to 50 for heathland shoots during the whole drought period (Fig. 2a – d). The C:N ratio of grassland shoots was significantly lower ($p = 0.01$) than that of heathland shoots. For all investigated shoots the C:N ratio increased ($p = 0.0001$) within the first 40 days of the drought and maximized for *C. vulgaris* and *V. myrtillus* during the drought phase I, whereas for grassland plant species (*P. lanceolata* and *H. lanatus*) the maximum value was observed after 70 days. After the maximum value, the C:N values of grassland and heathland shoots remained constant until the end of the drought phase III. For belowground biomass, the C:N ratio of heathland roots was significantly higher ($p = 0.001$) compared to that of grassland roots after 30 days (Fig. 2e – f). The C:N ratio reached the maximum (80.4 ± 3.68) for heathland roots ($p = 0.0001$) within the first 40 days of the drought and further decreased significantly ($p = 0.001$) during drought phase II (after 40 – 70 days). For grassland roots the C:N ratio did not reveal a significant trend during the whole drought experiment. After irrigation, the C:N ratio of grassland shoots returned to the same values that were observed during the begin of the drought experiment. A similar trend was observed for *V. myrtillus* (heathland shoots). However, the C:N ratios for the other heathland plant *C. vulgaris* didn't return to the pre-drought level. For belowground biomass, the C:N values of heathland roots remained significantly higher ($p = 0.001$) even 30 days after irrigation, compared to the latest phase of the

drought period, whereas no change was observed for grassland roots. In contrast to the shoots and roots, no significant trend was observed for C:N ratio of grassland and heathland soil (Fig. 2g – h), which ranged between 13 – 20 during the complete experimental drought period.

3.2. Stable isotope composition ($\delta^{13}C$) in unlabelled plots

For unlabelled plots, the $\delta^{13}C$ values of the shoots ranged between -30.0 and -27.7 ‰ at the beginning of the drought experiment (Fig 3a – d). Higher (p = 0.001) $\delta^{13}C$ values were observed for heathland shoots (*C. vulgaris*) compared to grassland shoots. During the first 10 days of drought, enriched ^{13}C values (2 – 3 ‰, p = 0.02) were determined for shoots for all investigated plants. During drought phases II and III, the $\delta^{13}C$ values for grassland shoots remained constant, whereas for heathland shoots, the $\delta^{13}C$ values returned to the initial level. The mean $\delta^{13}C$ values were almost identical for grassland roots (-29.7 ‰ ± 0.4 ‰) and heathland roots (-29.8 ‰ ± 0.3 ‰; Fig 3e – f). Within 40 days of the drought period the $\delta^{13}C$ values in grassland and heathland roots increased by ca. 2 ‰ (p = 0.02) and remained constant during the drought phase III. After irrigation, the $\delta^{13}C$ values returned back to their initial values for all shoot and root samples, except for heathland roots. Heathland soil was 0.5 ‰ more enriched in ^{13}C compared to grassland soil (Fig 3g – h), the difference not being significant (p = 0. 2). Similar to shoots and roots, the $\delta^{13}C$ values increased (<2 ‰) in soils during the first 10 days of the drought. After irrigation, the $\delta^{13}C$ values of soils returned to the initial level.

3.3. Above- and belowground carbon allocation in the labelled plots under drought

One day after the first $^{13}CO_2$ pulse labelling, the ^{13}C excess in grassland and heathland shoots ranged from 0.03 to 0.06 g of ^{13}C m^{-2}, indicating significant (p = 0.0001) assimilation of ^{13}C compared to natural ^{13}C background levels in unlabelled plots (Fig. 4a – d). Heathland shoots (*V. myrtillus* and *C. vulgaris*) revealed higher (p = 0.0001) ^{13}C excess compared with grassland shoots (*P. lanceolata* and *H. lanatus*). Strong fluctuations were determined for the amount of heathland biomass, especially of *C. vulgaris,* during the experiment on 800 cm^2 subplots of the 2 m^2 plots which is related to the large shrub type plants, of which the biomass was not equally distributed on the plots. This resulted in unequal portions of the biomass harvested for the different time intervals, thus leading to underestimation of the biomass for some sampling campaigns and subsequently overestimation of ^{13}C uptake. Therefore, 0.93 ± 0.3 g of ^{13}C m^{-2} can be used only as an approximation for the ^{13}C excess of the heathland plant *C. vulgaris.* After the second pulse labelling during drought phase II (44/45 days of drought), all plants showed significantly lower (p = 0.0001) ^{13}C uptake compared with the first labelling. The only exception was *P. lanceolata* (0.03 ± 0.0 g of ^{13}C m^{-2}), which did not reveal different ^{13}C excess in shoots compared with the first labelling. No significant ^{13}C uptake was observed after the third pulse labelling during the drought phase III (70/71 days

of drought) for most species, while *P. lanceolata* showed still showed a considerable ^{13}C uptake (0.02 ± 0.00 g of ^{13}C m^{-2}), which was in a similar order of magnitude compared to the first pulse labelling. Shoots of all investigated four plants species demonstrated a rapid initial ^{13}C tracer uptake after labelling, followed by a decline during the following weeks. Since no $^{13}CO_2$ pulse labelling was applied during the irrigation phase, ^{13}C tracer uptake by plants could not be determined after irrigation. However, the plots that received the second and third $^{13}CO_2$ pulse labelling were also analysed throughout the irrigation phase, but did not show any ^{13}C re-allocation in shoot biomass.

The results from freshly assimilated ^{13}C excess from shoots towards roots during the first labelling revealed that higher (p = 0.0001) ^{13}C amount was allocated from grassland shoots towards grassland roots compared to that for heathland (Fig. 4e – f). The maximum value of ^{13}C excess (0.015 ± 0.00 g m^{-2}) was observed for grassland roots 40 days after the first $^{13}CO_2$ pulse, whereas for heathland roots maximum tracer (0.01 ± 0.00 g m^{-2}) accumulation was observed within the first 10 days. The maximum relative allocation of ^{13}C tracer in grassland roots vs. shoots occurred 40 days after the first labelling (supplementary information S2). During the drought phase II, the second ^{13}C pulse labelling exhibited the maximum ^{13}C excess 15 days after in grassland roots (0.0082 ± 0.00 g m^{-2}), which was significantly higher than that of heathland roots (0.0059 ± 0.00 g m^{-2}). Almost negligible amount of ^{13}C enrichment was observed after the third isotope pulse labelling during severe drought for both roots of the investigated plant communities. After irrigation, in subplots that received the isotope pulse during drought phase II, we observed an increase in ^{13}C excess for grassland and heathland roots, respectively. Interestingly, the third $^{13}CO_2$ pulse labelling during the drought phase III also revealed a relative re-allocation of ^{13}C excess for roots of grassland and heathland ecosystems.

For soil, higher (p = 0.0001) ^{13}C enrichment was observed in heathland compared to grassland soil 40 days after the first ^{13}C labelling (Fig. 4 g – h). Similar like for shoots and roots, ^{13}C tracer allocation became subsequently lower for soil during phases II and III, i.e. after the second and third $^{13}CO_2$ pulse, respectively. Due to the very low ^{13}C tracer uptake in shoots and roots after the third pulse, allocation of ^{13}C in soil was lower for grassland and heathland roots and soil compared to the first pulse labelling. Surprisingly, within 30 days after irrigation ^{13}C enrichment for heathland soil returned to the maximum level of ^{13}C excess (0.0002 ± 0.00 g m^{-2}), which was observed 30 days after the second $^{13}CO_2$ pulse labelling. Such increase in ^{13}C excess was not observed for grassland soil after irrigation. Interestingly, heathland soil that received the third isotope pulse also revealed ^{13}C tracer enrichment within 10 – 15 days after irrigation.

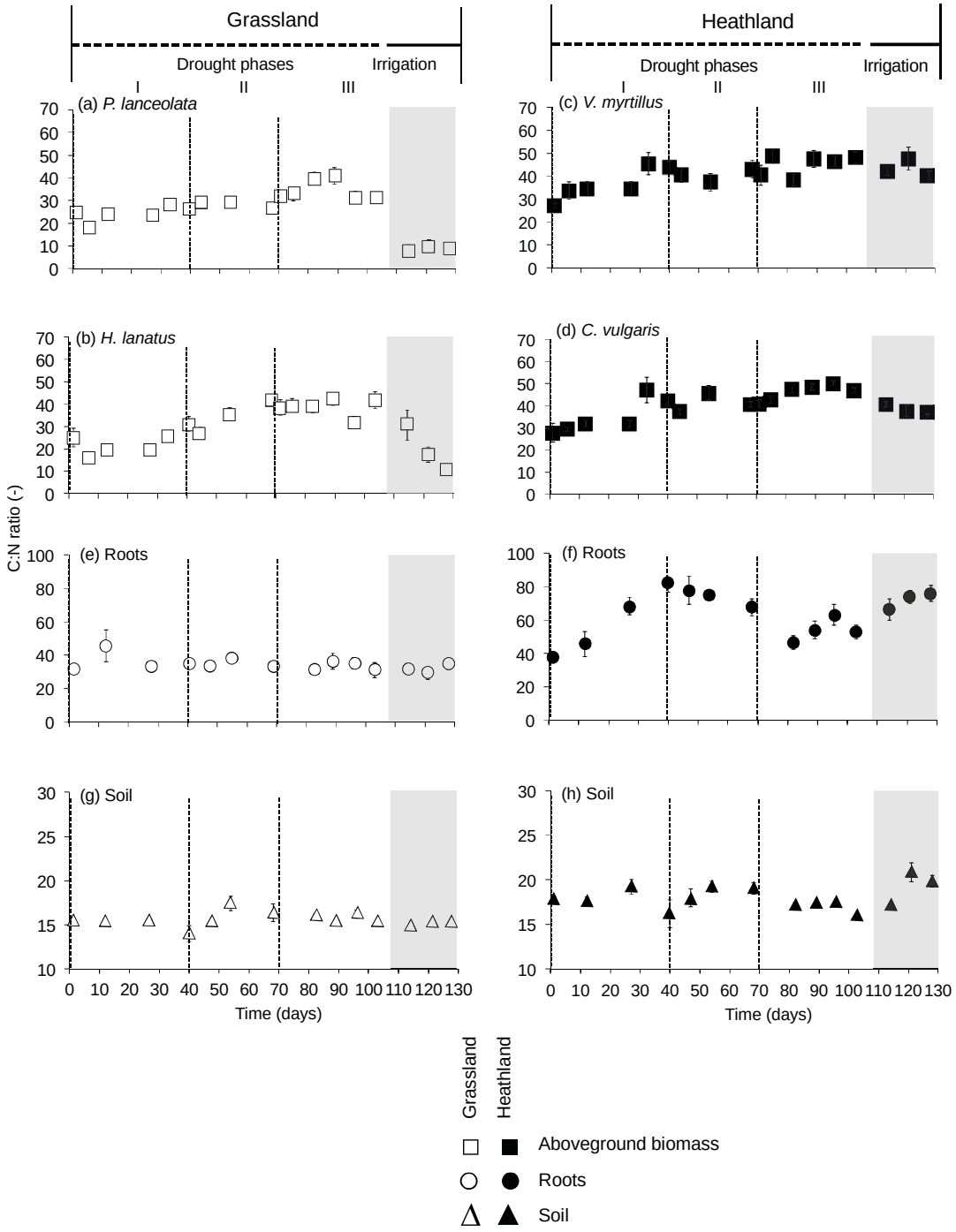

Figure 2. Time series analysis on the effect of severe drought on C:N ratios for shoots (a – d), roots (e – f) and soils (g – h) of a model grassland and heathland ecosystem. Mean and standard error of mean are given (field replicates, n = 10). Data point of day "0" represents control. Different drought phases are shown by a dotted line and divided into three phases (I, II and III). Irrigation treatment is illustrated by a solid line and shaded grey boxes.

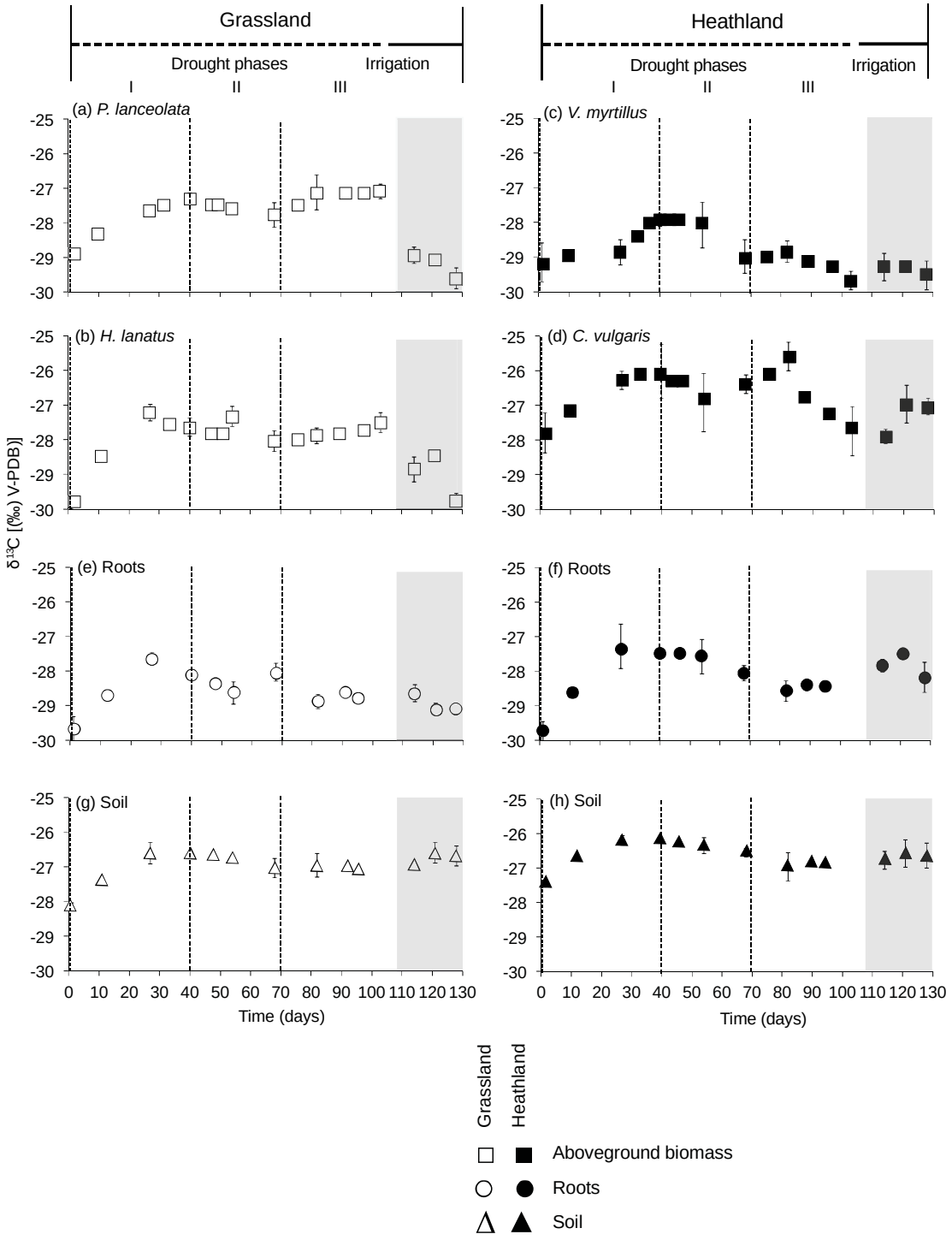

Figure 3. Time series analysis on the effect of severe drought on $\delta^{13}C$ for shoots (a – d), roots (e – f) and soils (g – h) of a model grassland and heathland ecosystem. Mean and standard error of mean are given (field replicates, n = 4). Data point of day "0" represents control. Different drought phases are shown by a dotted line and divided into three phases (I, II and III). Irrigation treatment is illustrated by a solid line and shaded grey boxes.

4. Discussion

4.1. Influence of severe drought on C cycling in the plant-soil system

4.1.1. Drought phase I (days 0 – 40)

The higher values of the C:N ratio under drought as observed for the aboveground biomass during the phase I of drought (Fig. 2a – h) is in agreement with previous drought studies conducted under field conditions [22,25,41]. It was expected to observe increased C:N ratios under drought due to increase in sugar accumulation and a decrease in leaf N content [25]. As expected for aboveground biomass, increased C:N ratios were more pronounced in heathland compared to grassland ecosystem [17,24]. The C:N ratios increase for heathland shoots within 40 days is most likely related to a decrease of available N in soil [22,25]. However, the C:N ratio did not significantly increase for grassland shoots most likely due to the presence of *L. corniculatus* (a leguminous plant), which fixed atmospheric N that was utilized by the plants during the first drought phase. Furthermore, allocation of C in roots to withstand drought led to increase in C:N ratios for roots [41]. For soil, the C:N ratio is an important parameter that describes the degradability of soil organic matter, which strongly depends on the soil type, vegetation and climate [17,24]. Due to the incorporation of dried litter and a subsequent microbial mineralization of easily degradable organic matter in soil, we expected an increase in the C:N ratio in the soil under drought [26,42]. Contrary to our expectation, we did not observe any significant change in the C:N ratio in soils exposed to the first 40 days of the drought period (Fig. 2g – h), which is logical when we take into account the big size of the soil organic matter pool and the slow turnover time of soil organic matter in a range of decades [6] and the lower input of plant-derived C in soils exposed to drought.

During the drought phase I, we expected to observe an increase in the $\delta^{13}C$ values of plant biomass under drought due to reduced stomatal conductance, which results in a selected ^{13}C enrichment in plant tissues [43,44]. According to our expectation, the $\delta^{13}C$ values increased during the initial 14 days of the drought for shoot biomass of all plant species (Fig. 3a – h). It is well documented that the isotopic signature can strongly differ between different plant parts [45,46]. However, in the current study the $\delta^{13}C$ values of the aboveground biomass represent a mixture of leaves and stems (*P. lanceolata* and *H. lanatus*) and partially also included woody branches (for *C. vulgaris* and *V. myrtillus*). In general, $\delta^{13}C$ values for all shoots remained within the typical range of C_3 plants and ranged between -27.2 ‰ to -29.5 ‰ during the drought phase I. (Fig 3 a – d) which is in agreement with previous observations [47,48]. Similar as for aboveground biomass, the $\delta^{13}C$ values of roots and soil were expected to increase with increasing drought

duration due to the subsequent transfer of ^{13}C-enriched assimilates from aboveground biomass towards roots and rhizosphere and ^{13}C-enrichment of soil organic matter related to microbial degradation of easily degradable components [17,48]. As expected, grassland and heathland roots revealed increased $\delta^{13}C$ values under drought, confirming previous observations [48]. Also more positive $\delta^{13}C$ values were obtained for grassland and heathland soils within the first 40 days of the drought. The fact that $\delta^{13}C$ values changed in soils is quite surprising if the C:N ratio is taken into account, which did not change. If the hypothesis would hold true that soil C is predominantly deriving from aboveground biomass, not only $\delta^{13}C$ values, but also C:N ratios of soils should have changed according to the drought-related changes of aboveground biomass. As roots and soil showed the same trends for $\delta^{13}C$ values and C:N ratios, which is different for aboveground biomass, our findings suggest that during the investigated drought period, roots and most likely microorganisms in the rhizosphere feeding on root exudates and root remains influence soil organic matter composition. This observation is in line with other studies highlighting the significance of root-derived organic matter [17,49], whereas such observations have been missing for C cycling in field studies, where model ecosystems are exposed to severe drought.

The first $^{13}CO_2$ labelling in the beginning of the drought period (day 0/1) showed a higher amount of ^{13}C tracer uptake for heathland shoots compared to grassland shoots (Fig 4 a – d). The ^{13}C tracer was significantly higher in the investigated shoots compared to roots and soil and gradually declined over time, which is a general pattern observed during $^{13}CO_2$ pulse-chase labelling experiments [16,31]. This decrease of ^{13}C in shoots over time is attributed to respired C and translocated C towards the root-soil compartment [50]. Additionally, this is accompanied by dilution due to plant uptake of atmospheric CO_2 with natural $\delta^{13}C$ composition [44,51]. The first labelling showed substantial C uptake by all investigated plants. The ^{13}C tracer uptake and translocation towards roots revealed the maximum ^{13}C excess within 10 days. This indicated that for heathland plants a comparatively large amount of assimilated C is allocated in roots already shortly after the labelling (Fig 4e – f). The second maximum after 40 days observed for allocated C in heathland roots was surprising. This argues for subsequent tracer allocation from shoot biomass towards root biomass due to improved biomass production in the root system during the initial drought for improved acquisition of water and nutrients [31,50], which is also confirmed by an increase of the ratio of root to shoot biomass under water stress [35]. In contrast to heathland, for grassland roots the maximum ^{13}C tracer excess was observed much later, i.e. 40 days after the first labelling [50]. The time-lag for C transfer from shoots towards roots can be related to the drought induced stress and therefore limited exchange of water, C and nutrients in grassland plants [16]. Furthermore, a subsequent increase in belowground ^{13}C suggested ongoing allocation of assimilated ^{13}C tracer in roots due to a time lag of C allocation in root vs. shoot biomass [50,52], where the long duration of the ^{13}C excess peak could result either from a very limited, but ongoing allocation of C towards roots [11,16], or probably we missed the

first peak of ^{13}C tracer excess shortly after the first isotope pulse due to the sampling scheme, where samples were taken in too long sampling intervals. For grassland roots, the tracer allocation pattern revealed high relative amounts of C was allocated towards the roots 40 days after the first labelling, which suggested a strong drought effect for grassland shoots therefore ^{13}C was allocated slowly from shoots to roots during the beginning of the drought where annual plants need to adapt with the drought period [11]. The maximum ^{13}C excess in grassland and heathland soil was observed also 40 days after the first labelling, which is in agreement with root results and argues for a subsequent incorporation of C due to improved root growth and release of root-derived organic matter during the first drought phase [27,50].

Especially during the initial drought phase I strong changes were observed in the plant-soil system as reflected by $\delta^{13}C$ values and C:N ratios through decreasing nutrient availability in already dried soils [24,53]. The fast uptake of ^{13}C tracer after first labelling in shoot biomass revealed very active plant biosynthesis, whereas the subsequent allocation of C towards roots and soil is already limited due to the limited water and nutrient mobilization during drought phase I [50].

4.1.2. ***Drought phase II (day 40 to day 70)***

We expected to observe further increase of the C:N ratio in the plant-soil system as intense drought can reduce the plant nutrient uptake through decreasing nutrient availability and reducing nutrient supply in dried soils [17]. However, no further increase in C:N ratios was observed in the shoots compared to first drought phase. A similar result was obtained for grassland roots whereas heathland roots revealed reduced C:N ratio under more intense drought most likely due to investment of more C for shoot biomass and reduced supply of C towards roots [11,14]. The C:N ratios of grassland and heathland soils increased during the drought phase II, which might be related to the fact that during this period decomposition of soil organic matter was strongly limited although plants were still active at this stage. Consequently, increased C allocation from plants towards soil led to enrichment of soil C, whereas N was continuously taken up by the plants. This subsequently increased the C:N ratio in grassland and heathland soil. [54].

During drought phase II, the $\delta^{13}C$ values of plant biomass and soil decreased for all samples. This indicates that intense drought led to a decrease in the C assimilation rate, which resulted in less C availability within some plant tissues [43,48] and subsequent isotope fractionation most likely due to release of ^{13}C-enriched biosynthesates [44]. The $\delta^{13}C$ values for grassland and heathland soil reflected the values observed in grassland and heathland shoots and roots, respectively [46]. The result of second $^{13}CO_2$ pulse labelling during the drought phase II revealed significantly lower ^{13}C excess in grassland and heathland shoots root and soil compared to the first labelling, although similar amount of ^{13}C tracer were

applied. This is due to the reduction in the C uptake and its allocation under extended drought [16,31]. Lower ^{13}C excess during second labelling is most likely due to almost dried aboveground biomass, which was visible on the experimental site already after 50 days of drought.

Due to sampling schedule of the current study, we missed the peak of ^{13}C excess for all root samples, but our sampling scheme enabled to identify the re-allocation ^{13}C as an effect of limited CO_2 uptake and therefore plant internal translocation of C. Therefore, the highest ^{13}C excess for grassland roots and also for heathland roots were observed 30 days after the second pulse labelling. This argues for subsequent ^{13}C tracer allocation in roots due to stronger investment in root systems during the drought phase II (40 – 70 days of drought). Compared to the first labelling, maximum ^{13}C excess of heathland roots showed a larger time-lag, i.e. we observed the highest value 30 days after the labelling. This can be associated to the fact that during the intense drought period, heathland plant invest more C for the formation and maintenance of the tissue to withstand under drought [11,16,55]. The heathland soil revealed maximum ^{13}C excess around 30 days after the second pulse labelling. For grassland soil the maximum ^{13}C excess occurred 45 days after the second pulse labelling. Thus, as expected, plant C was still allocated into the soils during the drought phase II.

4.1.3. ***Drought phase III (day 70 to day 104)***

The drought phase III is considered as the most intense severe drought period. During this phase, plants from grassland and heathland communities were remarkably stressed. In all investigated plots, yellow to brown leaves (smaller than the normal size), defoliation and dried twigs indicated that aboveground biomass was not active at this phase. Therefore, we expected no further increase in C:N ratio in the plant biomass. However, both grassland plants (*P. lanceolata* and *H. lanatus*) showed increased C:N ratio most likely due to ongoing degradation of N-containing compounds in plants such as amino acids and amino sugars as well as translocation of N towards roots (after 80 days) as previously suggested [10,54]. We expected to observe high C:N ratios for roots under drought, due to limited nutrient supply, which was observed only for heathland roots. Contrary to our expectation, grassland roots did not change their C:N ratio under drought, which argues for limited biosynthetic activity. Soil C:N ratio did not show any change after 70 days of the drought period for both communities. As significant changes in shoot C:N ratios were observed even during the severe drought phase, but not for roots and soils, this suggested that the severe drought potentially weakened the coupling of above- and belowground C and N dynamics in the investigated plant-soil systems. In other words, similar trends in roots and soils indicate the significance of root biomass regulating soil organic matter composition, whereas aboveground biomass did not significantly influence soil organic matter during the latest drought phase.

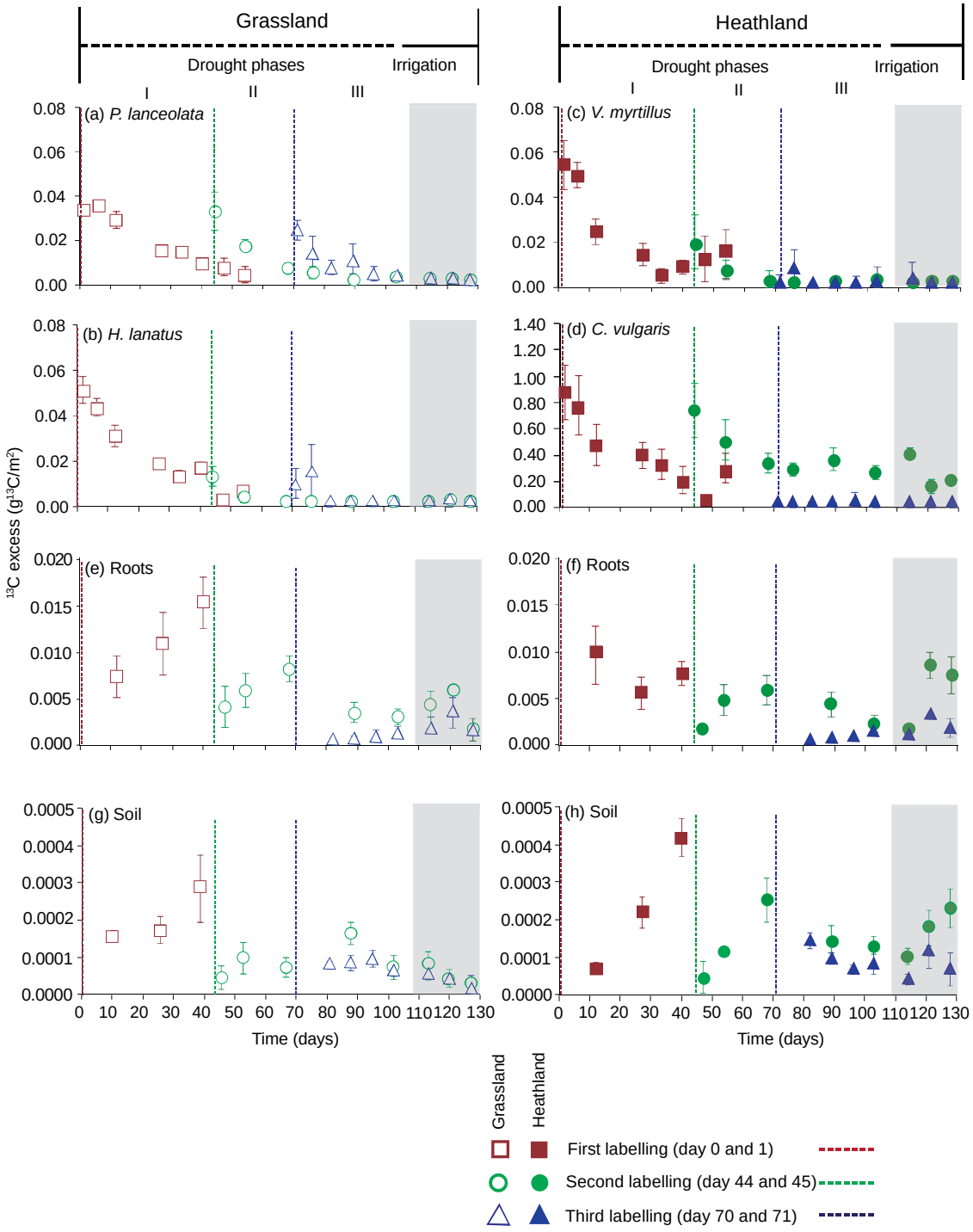

Figure 4. ^{13}C excess (g m^{-2}) in shoots (a – d), roots (e – f) and soils (g – h) of a model grassland and heathland ecosystems. $^{13}CO_2$ pulse labelling is indicated by dotted line three times in three different colors on day 0/1, 44/45 and 70/71 in grassland and heathland respectively. The mean and standard error of mean are given (field replicates, n = 6). Different drought phases are shown by a dotted line and divided into three phases (I, II and III). Irrigation treatment is illustrated by a solid line and shaded grey boxes.

After 70 days of drought, the $\delta^{13}C$ values of unlabelled grassland shoots (*P. lanceolata* and *H. lanatus*) showed no change whereas, for heathland shoots, (*C. vulgaris* and *V. myrtillus*) the $\delta^{13}C$ values returned back to the pre-drought level. The shift in heathland $\delta^{13}C$ is associated to the increasing ratio of woody to non-woody biomass during ongoing drought [48]. Grassland plants were not able to resist against severe drought compared to heathland plants which might resist the drought period by maintaining a C balance by their woody tissues [14,56]. The $\delta^{13}C$ values for grassland and heathland roots and soil revealed similar trends as the respective shoot biomass.

After the third $^{13}CO_2$ isotope pulse no significant ^{13}C uptake, assimilation and incorporation of the tracer were observed in shoots, roots and soil for heathland ecosystems. For grassland shoots, interestingly, *P. lanceolata* demonstrated high uptake of ^{13}C compared to other investigated plants even after 70 days of the severe drought period. Compared to the first pulse labelling, the ^{13}C excess in shoots and its incorporation in roots and soil were significantly decreased during the third pulse labelling experiment. The observed reduction in ^{13}C uptake after 80 days of the drought could not be simply verified with other studies due to its very long duration of the drought period, which was not common to simulate in field studies.

Hence, in line with our first hypothesis, the observed reduction in ^{13}C uptake in shoots from the initial drought to severe drought suggested that with increasing drought the plant C uptake and the proportion of freshly assimilated ^{13}C becomes lower, thus confirming that plant C uptake decreased with increase in drought period and also C allocation from shoots towards roots and soil decreased exponentially over time as observed during three different phases of the severe drought period.

4.2. Effect of irrigation after severe drought on C cycling in the plant-soil system

To understand how plant C allocation was re-activated in the plant-soil system directly after the severe drought, the current study monitored plant C re-allocation and incorporation in soil during the irrigation phase after a severe drought. After irrigation, the C:N ratios of grassland and heathland shoots decreased within 10 days indicating the rapid regeneration of plant growth and uptake of nutrients, which was faster for grassland than for heathland plants [7,8]. Due to irrigation, C:N ratios of shoots and roots returned to pre-drought level for grassland and heathland ecosystems within 10 – 15 days. However, no change was observed for the C:N ratio of grassland soil. The missing change of the grassland soil C:N ratio after irrigation can be mainly related to two reasons: (a) Due to the comparatively low change in plant C:N ratios, especially for root biomass, significant changes in soil C:N ratios are not likely to be expected. (b) The large C and N stocks in soil are typically not significantly influenced in the short-term, which is related to the low turnover of C in the range of decades [6].

The irrigation after the severe drought was expected to increase the stomatal conductance of leaves again, which consequently should lead to decrease of the $\delta^{13}C$ value in aboveground biomass and the whole plant-soil system [57]. As expected, $\delta^{13}C$ values rapidly decreased in grassland shoots, roots and soil after irrigation. This suggested a close coupling between C cycling in above- and belowground biomass and suggested that temperate grassland plants have potential to rapidly compensate the severe drought through regulating their photosynthetic processes, renewal of plant biomass and restarting of the allocation of C within (10 – 15 days) a short time period [7,8]. Also heathland shoots showed decreased $\delta^{13}C$ value after irrigation. However, contrary to our expectation, heathland roots revealed 2.5 ‰ enrichment in ^{13}C after irrigation, which might be attributed to the fact that heathland plants are conservative in nature in terms of their C investment and stored C in the tissues for longer time period [55,58].

Sampling after the second and third $^{13}CO_2$ pulse labelling experiment was continued also after irrigation. Freshly assimilated re-allocation of ^{13}C from shoots to roots after irrigation demonstrated that re-activation of C cycling occurred. This pattern was also observed for grassland and heathland soils. This is most likely due to re-activation of C allocation from shoots to roots and thus incorporation of ^{13}C tracer into roots and soils. Also for the grassland community, the irrigation significantly affected grassland roots, where ^{13}C excess was traced in roots within 10-15 days after irrigation [8,59]. In line with our second hypothesis, the C re-allocation was observed for roots and soil in grassland and heathland just within 15 days after irrigation. This suggested the C, which was assimilated and stored in the aboveground biomass during the severe drought, was further re-allocated towards the belowground biomass and soil, as a result of re-activation of C cycling in the plant-soil system. This is related to the rapid regeneration of the aboveground biomass [7,8] and preferential C assimilation, allocation and incorporation of labelled ^{13}C on which soil microorganisms are feeding on, consequently leading to high enrichment of ^{13}C in roots and soil [42,60].

4.3. ***Comparison of C cycling in grassland vs. heathland plant-soil system exposed to severe drought followed by irrigation***

The current study provides a comparison of ^{13}C uptake and assimilation within shoots and roots of grassland and heathland communities during the phases of increasing drought followed by irrigation. Significant differences occurred between grassland and heathland shoot ^{13}C tracer uptake and its allocation towards roots and soil.

The first labelling results revealed that the ^{13}C tracer uptake by shoots (Fig. 4 a – d) and its allocation towards roots was very fast in heathland, maximizing 10 days after the isotope pulse [50,55,58]. This

indicated that for heathland plants a comparatively large amount of assimilated C is allocated towards roots (Fig 4e – f) which is in agreement with previous studies [56,58,61]. In contrast to heathland, for grassland roots the maximum relative allocation of ^{13}C tracer was observed much later, i.e. 40 days after the first labelling [50]. The time-lag for C allocation from shoots towards roots in grassland was expected to be shorter compared to the heathland plants during the first labelling because this speed of C allocation depends on the plants size [62]. As also for heathland roots a second maximum was observed 40 days after the first isotope pulse, this suggests that for both ecosystems during the drought phase I, C was subsequently re-allocated from shoots to roots to improve root growth under drought [58]. For soil (Fig. 4g – h), higher ^{13}C excess for heathland compared to grassland was observed 40 days after the first labelling, which was very likely caused by higher amount of C in stored in shoots and roots of heathland community, whereas for grassland larger amount of the tracer was respired more rapidly [55,58].

During the second pulse labelling in drought phase II, all plants were still actively assimilate the applied $^{13}CO_2$ tracer, but C uptake and its allocation towards roots and soil was significantly lower compared to the first isotope pulse labelling. C allocation was higher in heathland compared to grassland soil, which can be also related to the larger amount of C stored in aboveground heathland biomass and less C respiration, as already described before [47,55] and confirming our third hypothesis. The third pulse labelling revealed no further significant ^{13}C tracer uptake for heathland shoots whereas unexpectedly the grassland plant *P. lanceolata* revealed C uptake even after 70 days of the drought. However, compared to the first pulse the ^{13}C excess was significantly lower after the third pulse. Roots of both communities did not show considerable ^{13}C allocation from shoots towards roots during the third pulse labelling. However, as an effect of irrigation, re-activation of C cycling was observed for grassland and heathland roots and soil within 15 days after irrigation. ^{13}C tracer that was incorporated in shoot biomass during the drought experiment was significantly higher in heathland soil, when compared to grassland soil. Furthermore, only shortly after irrigation tracer allocation increased in grassland soil, whereas for heathland soil a subsequent increase of C allocation was determined until the end of the observation period. Overall, this argues for incorporation of large tracer amount into woody heathland aboveground biomass, which was used as a C reservoir after re-activation of C cycling in the plant-soil system after irrigation[7,47,55]. For the grassland ecosystem, storage of C in shoot biomass was less important arguing for either improved C allocation in roots and soil, or respiration of C by shoot biomass [56,58]. As C allocation in roots and soil was comparable in heathland and grassland roots and soil, higher C respiration in the model grassland ecosystem vs. higher C storage in aboveground biomass in heathland can be assumed to be the most dominant factors explaining the observed drought-induced response of C cycling in these ecosystems [16,29].

5. Conclusions

Ongoing climate change raises questions about the response of C cycling and the significance of above- vs. belowground processes in temperate plant-soil systems exposed to the extended drought duration. In the current study, model temperate grassland and heathland ecosystems were exposed to 104 days of drought followed by irrigation. The current study revealed that (1) C:N ratios in the heathland plant-soil system increased after 40 days of drought and for grassland C:N ratios maximized after 70 days of drought. (2) Plant C uptake and its assimilation and allocation decreased with ongoing duration of the drought period. (3) Only 10–15 days after irrigation after the severe drought, C that was incorporated and stored in the shoots during the drought was re-allocated in the roots and soil as soon as irrigation started. Severe drought period induced a strong effect on the short-term C cycling in the plant-soil system. The different trends of C allocation pattern for grassland compared to heathland roots suggest that plant responses to drought cannot be generalized and necessitate further investigation across different ecosystems. In the current study, higher C storage in heathland led to higher C allocation towards soil for the heathland ecosystem. Further investigations are required on extended drought periods followed by irrigation phases in other biomes in order to draw general conclusion and to answer the question, how fast the complete C cycle in the grassland and heathland ecosystem can recover after severe drought.

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Part C

Appendix

Supplementary data (Synopsis)

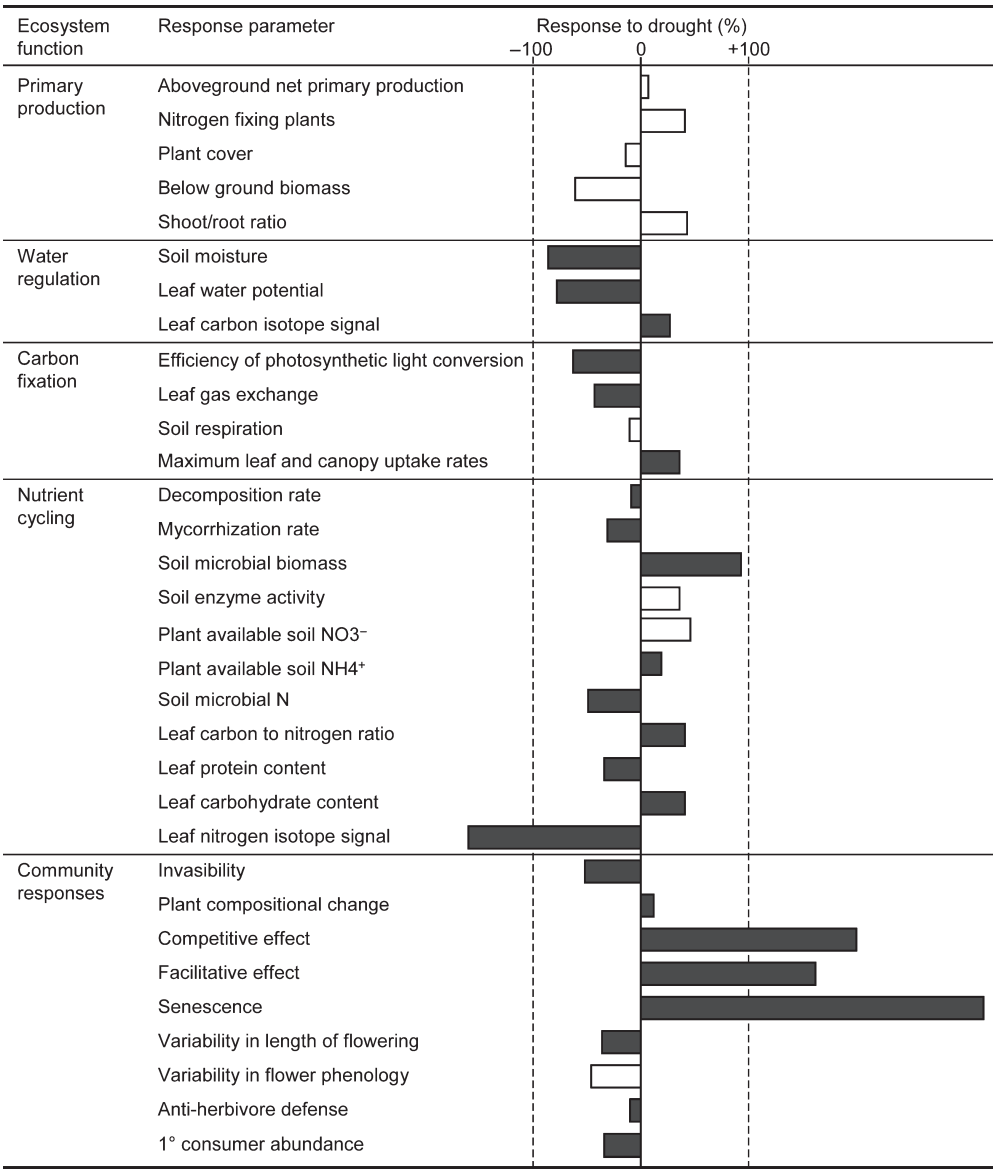

Figure S.1: Effects of recurrent annual drought events on 32 response parameters organized into ecosystem functions. All data were collected at the EVENT-I experimental site during the years 2005–2009. A parameter is marked as significant (filled black bar) if data of at least 1 year showed significant differences between drought and ambient conditions. Data shown represent maximum effects from years with highest drought effects, averaged over all three experimental grassland communities. Adopted from Figure 1, Jentsch *et al.* (2011)

Supplementary data (Manuscript I)

Table S.1: Relative abundance of integrated *n*-alkanes (*n*-C_{19} to *n*-C_{33}) shown in percentage (%) in shoots, roots and soil of grassland and heathland model ecosystem under control and one year after repeated annual drought conditions. Values (mean ± SE, n = 5) are given as the mean with the standard errors of five field replicates.

Model ecosystem	Sample type	Plant species	Treatment	*n*-C_{19}	*n*-C_{20}	*n*-C_{21}	*n*-C_{22}	*n*-C_{23}	*n*-C_{24}	*n*-C_{25}	*n*-C_{26}	*n*-C_{27}	*n*-C_{28}	*n*-C_{29}	*n*-C_{30}	*n*-C_{31}	*n*-C_{32}	*n*-C_{33}
Grassland	Aboveground biomass	*P. lanceolata*	Control	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.9 ± 0.5	0.6 ± 0.2	11.4 ± 6.1	2.2 ± 1.0	32.7 ± 10.2	2.6 ± 1.3	37.6 ± 11.7	1.9 ± 1.0	8.6 ± 4.3
			Drought	0.3 ± 0.3	0.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	6.2 ± 4.2	0.5 ± 0.4	5.8 ± 4.1	0.4 ± 0.3	8.4 ± 6.6	0.3 ± 0.3	13.3 ± 6.7	3.2 ± 0.7	38.9 ± 14.0	3.1 ± 1.3	9.6 ± 3.6
		H. lanatus	Control	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.4	13.0 ± 0.1	6.6 ± 4.1	0.0 ± 0.0	6.9 ± 3.0	4.6 ± 2.1	9.3 ± 3.7	1.0 ± 0.5	43.4 ± 13.1	6.9 ± 4.1	14.5 ± 7.1	0.5 ± 0.5	1.8 ± 1.5
			Drought	0.0 ± 0.0	0.1 ± 0.0	0.9 ± 0.5	0.3 ± 0.1	7.1 ± 4.1	0.2 ± 0.1	7.3 ± 3.7	0.5 ± 0.3	13.9 ± 2.4	0.7 ± 0.3	43.1 ± 15.5	4.0 ± 0.8	14.9 ± 7.9	1.3 ± 0.6	5.0 ± 2.7
		L. corniculatus	Control	0.6 ± 0.3	0.6 ± 0.3	0.2 ± 0.1	5.6 ± 2.9	0.8 ± 0.1	3.5 ± 1.9	2.6 ± 1.8	2.3 ± 0.9	20.0 ± 2.1	1.9 ± 0.3	24.9 ± 7.0	2.2 ± 0.4	23.6 ± 3.6	1.2 ± 0.6	9.1 ± 3.1
			Drought	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.5	0.1 ± 0.0	9.0 ± 5.4	0.8 ± 0.3	16.9 ± 2.0	1.4 ± 0.3	26.3 ± 5.4	1.5 ± 0.2	15.2 ± 2.9	0.5 ± 0.2	17.1 ± 0.2	1.2 ± 0.1	8.7 ± 1.9
		Avg. aboveground biomass	Control	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	6.3 ± 1.1	2.5 ± 2.2	1.2 ± 0.7	3.5 ± 1.4	2.5 ± 1.0	13.6 ± 4.0	1.7 ± 0.6	36.7 ± 10.0	3.9 ± 1.8	25.2 ± 7.2	1.2 ± 0.9	6.5 ± 3.2
			Drought	0.1 ± 0.1	0.3 ± 0.2	0.6 ± 0.3	0.1 ± 0.0	7.4 ± 4.5	0.5 ± 0.3	10.0 ± 3.8	0.8 ± 0.3	16.2 ± 4.8	0.9 ± 0.3	23.9 ± 8.2	2.6 ± 0.6	23.6 ± 7.5	1.9 ± 0.6	7.8 ± 2.8
	Roots		Control	0.2 ± 0.0	2.3 ± 0.6	4.9 ± 1.4	2.3 ± 0.6	4.5 ± 1.0	3.5 ± 1.2	16.8 ± 4.3	2.9 ± 1.6	15.6 ± 4.1	6.5 ± 1.0	18.9 ± 1.1	2.4 ± 0.7	12.0 ± 2.6	1.6 ± 0.9	3.9 ± 1.3
			Drought	0.0 ± 0.0	0.4 ± 0.0	2.5 ± 0.8	2.0 ± 0.9	3.9 ± 1.2	5.6 ± 1.6	14.4 ± 1.9	2.9 ± 0.8	19.2 ± 0.6	6.1 ± 0.7	19.1 ± 1.3	2.4 ± 1.0	10.6 ± 3.0	1.0 ± 0.5	1.9 ± 0.7
	Soil		Control	0.1 ± 0.0	1.0 ± 0.1	3.6 ± 0.4	0.8 ± 0.3	4.9 ± 0.2	1.8 ± 0.3	12.5 ± 0.6	2.7 ± 0.1	19.6 ± 0.7	5.0 ± 0.1	19.7 ± 0.4	2.4 ± 0.3	15.5 ± 1.0	0.9 ± 0.1	4.7 ± 0.4
			Drought	0.8 ± 0.4	1.2 ± 0.1	3.1 ± 0.2	0.5 ± 0.2	4.9 ± 0.2	2.3 ± 0.1	11.5 ± 2.7	2.7 ± 0.3	18.7 ± 0.5	4.5 ± 0.2	19.9 ± 0.4	2.6 ± 0.4	16.7 ± 0.4	1.6 ± 0.1	4.9 ± 0.3
Heathland	Aboveground biomass	*V. myrtillus*	Control	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	7.3 ± 1.2	0.6 ± 0.1	18.2 ± 1.6	1.9 ± 0.1	27.1 ± 2.9	2.1 ± 0.6	33.3 ± 2.7	1.2 ± 0.6	6.5 ± 3.0
			Drought	0.0 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	7.3 ± 1.5	1.4 ± 0.5	14.4 ± 1.1	3.0 ± 0.9	25.5 ± 1.6	2.1 ± 0.4	39.4 ± 3.1	0.9 ± 0.0	4.2 ± 0.5
		C. vulgaris	Control	0.1 ± 0.0	0.2 ± 0.0	1.9 ± 0.4	0.3 ± 0.0	1.7 ± 0.1	0.4 ± 0.0	2.0 ± 0.1	1.0 ± 0.3	11.3 ± 0.6	3.0 ± 0.1	21.9 ± 0.9	3.8 ± 0.2	24.5 ± 0.9	5.0 ± 0.4	12.9 ± 6.9
			Drought	0.0 ± 0.0	0.1 ± 0.0	0.9 ± 0.1	0.2 ± 0.0	1.1 ± 0.2	0.3 ± 0.0	2.0 ± 0.2	1.2 ± 0.2	11.3 ± 1.4	2.5 ± 0.3	20.3 ± 2.7	3.0 ± 0.4	37.3 ± 4.2	4.0 ± 0.8	15.1 ± 5.0
		Avg. aboveground biomass	Control	0.2 ± 0.1	0.2 ± 0.0	1.1 ± 0.3	0.2 ± 0.0	1.0 ± 0.1	0.3 ± 0.0	4.7 ± 0.8	0.8 ± 0.2	14.7 ± 1.7	2.4 ± 0.1	24.5 ± 1.9	3.0 ± 0.4	28.9 ± 1.9	3.1 ± 0.6	9.7 ± 5.0
			Drought	0.0 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	0.9 ± 0.1	0.3 ± 0.0	4.7 ± 1.0	1.3 ± 0.3	12.8 ± 1.3	2.7 ± 0.6	22.9 ± 2.0	2.6 ± 0.3	38.3 ± 3.6	2.5 ± 0.5	9.6 ± 2.8
	Roots		Control	0.0 ± 0.0	0.6 ± 0.5	6.3 ± 3.7	0.6 ± 0.6	7.6 ± 2.4	1.3 ± 1.0	8.9 ± 1.1	0.6 ± 0.4	6.9 ± 3.0	1.3 ± 0.8	11.5 ± 1.40	6.3 ± 3.7	26.8 ± 6.8	5.6 ± 4.3	13.4 ± 2.7
			Drought	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 2.0	8.0 ± 4.6	0.0 ± 0.0	5.0 ± 4.5	0.0 ± 0.0	40.0 ± 1.0	0.0 ± 0.0	10.0 ± 1.0
	Soil		Control	0.2 ± 0.0	1.3 ± 0.1	4.3 ± 0.2	1.7 ± 0.6	6.1 ± 0.6	2.5 ± 0.2	10.6 ± 0.2	2.1 ± 0.1	15.5 ± 0.7	2.3 ± 0.2	13.8 ± 0.5	1.9 ± 0.1	20.1 ± 0.6	1.2 ± 0.1	10.2 ± 1.5
			Drought	0.3 ± 0.0	0.7 ± 0.2	3.0 ± 0.8	0.2 ± 0.1	4.6 ± 1.2	1.8 ± 0.5	7.6 ± 1.1	1.7 ± 0.4	12.0 ± 3.0	2.1 ± 0.5	14.5 ± 0.3	1.5 ± 0.3	22.2 ± 0.6	1.3 ± 0.3	12.9 ± 0.5

Table S.2: Stable carbon isotope composition ($\delta^{13}C$) shown in per mil (‰) in shoots, roots and soil of grassland and heathland model ecosystem under control and one year after 5 years of repeated annual drought conditions[a]

Model ecosystem	Sample type	Plant species	Control					Drought				
			Plots[b]									
			27/GC4+1	**44/CG4+2**	**69/CG4+3**	**118/CG4+4**	**132/CG4+5**	**10/DG4+1**	**59/DG4+2**	**75/DG4+3**	**113/DG4+4**	**125/DG4+5**
Grassland	Aboveground biomass	*P. lanceolata*	-29.40	-29.02	-29.18	-29.25	-29.66	-29.48	-28.77	-28.79	-29.75	-29.36
		H. lanatus	-30.14	-30.38	-30.23	-29.75	-30.20	-30.46	-30.77	-30.07	-30.74	-30.89
		L. corniculatus	-28.40	-29.02	-29.84	-29.53	-29.79	-27.29	-29.18	-28.57	-28.10	-29.29
	Roots		-29.60	-30.91	-29.04	-30.59	-30.65	-29.65	-31.42	-28.73	-29.59	-30.92
	Soil		-27.54	-28.23	-28.26	-28.10	-28.29	-28.14	-28.20	-27.84	-28.36	-28.19
			25/CH2-1	**46/CH2-2**	**67/CH2-3**	**119/CH2-3**	**141/CH2-5**	**11/DH2-1**	**60/DH2-2**	**74/DH2-3**	**98/DH2-4**	**126/DH2-5**
Heathland	Aboveground biomass	*C. vulgaris*	-28.31	-28.36	-28.77	-27.57	-27.26	-26.67	-27.17	-27.33	-26.60	-27.91
		V.myrtillus	-30.72	-30.36	-30.52	-30.54	-30.73	-28.25	-30.14	-30.37	-29.91	-30.56
	Roots		nd	-29.37	nd	nd	-29.12	nd	-30.04	nd	nd	-30.67
	Soil		-27.99	-27.41	-27.90	-27.29	-27.28	-27.36	-27.42	-27.35	-27.32	-27.40

[a] nd, *not detected*,

[b] C = control, D = drought, G = grassland, H = heathland, 2/4 = number of species, 1-5 = plot rows.

Supplementary data (Manuscript II)

Table S.3: ACL, CPI and relative abundance (%) of long-chain *n*-alkanes (n-C_{25}–n-C_{35}) in grassland and heathland ecosystems exposed drought. Mean ± standard errors of the mean are given (measurement replicates, n = 10). Data point on day '0' represents control.

Ecosystem type	Sample type	Drought phase	Sampling days	ACL (n-C_{25-35})	CPI (n-C_{25-35})	n-C_{25+27} (%)	n-C_{29} (%)	n-C_{31+33} (%)
Grassland	*H. lanatus*	I	0	29.0 ± 0.2	11.2 ± 0.8	39.2 ± 3.0	22.5 ± 2.8	38.1 ± 5.4
			12	28.4 ± 0.2	10.2 ± 0.8	44.2 ± 4.2	32.1 ± 1.4	23.6 ± 3.0
			27	29.2 ± 0.1	12.0 ± 0.7	26.2 ± 1.3	38.2 ± 0.9	38.2 ± 1.3
		II	40	29.3 ± 0.2	11.5 ± 0.8	28.4 ± 2.8	37.5± 3.2	37.5 ± 5.7
			54	29.1 ± 0.0	13.0 ± 0.3	26.1 ± 0.7	42.6 ± 1.0	42.6 ± 1.0
			68	29.2 ± 0.0	12.8 ± 0.4	25.9 ± 1.3	40.5 ± 1.3	40.6 ± 1.3
		III	82	29.4 ± 0.1	14.0 ± 1.0	23.0 ± 1.9	40.4 ± 2.5	40.4 ± 3.4
			96	28.9 ± 0.0	11.4 ± 0.6	28.7 ± 0.3	41.1 ± 0.8	41.1 ± 0.9
			103	29.4 ± 0.2	11.6 ± 0.5	21.3 ± 2.3	37.9 ± 3.5	37.9 ± 5.8
	Roots	I	0	28.3 ± 0.1	4.5 ± 0.2	48.2 ± 2.3	25.8 ± 2.9	26.0 ± 2.9
			12	29.1 ± 0.2	4.0 ± 0.3	34.2 ± 1.7	28.4 ± 5.4	37.4 ± 6.3
			27	29.1 ± 0.1	4.4 ± 0.2	32.1 ± 2.9	32.7 ± 1.0	35.2 ± 3.5
		II	40	28.5 ± 0.2	3.7 ± 0.3	42.7 ± 3.2	31.5 ± 2.0	25.8 ± 3.3
			54	29.8 ± 0.3	4.4 ± 0.4	24.5 ± 3.7	27.1 ± 3.0	48.4 ± 5.5
			68	29.0 ± 0.2	4.7 ± 0.2	32.2 ± 3.2	35.1 ± 2.0	32.6 ± 4.9
		III	82	29.4 ± 0.2	4.1 ± 0.4	30.3 ± 3.9	28.0 ± 2.2	41.7 ± 2.7
			96	29.5 ± 0.2	3.7 ± 0.4	29.5 ± 7.0	31.7 ± 1.9	38.8 ± 8.8
			103	29.6 ± 0.2	4.2 ± 0.4	24.5 ± 3.7	25.3 ± 2.7	49.3 ± 5.6
	Soil	I	0	28.5 ± 0.0	5.0 ± 0.1	43.4 ± 1.0	27.5 ± 0.3	29.1 ± 0.9
			12	29.6 ± 0.0	6.7 ± 0.1	25.8 ± 0.7	27.5 ± 0.5	46.6 ± 0.9
			27	29.8 ± 0.0	8.1 ± 0.3	22.3 ± 0.6	26.0 ± 0.8	51.6 ± 1.0
		II	40	29.6 ± 0.1	6.2 ± 0.1	25.3 ± 1.5	26.3 ± 0.6	48.4 ± 1.2
			54	29.8 ± 0.1	7.5 ± 0.2	23.2 ± 1.5	26.1 ± 1.5	50.7 ± 3.0
			68	30.0 ± 0.1	7.9 ± 0.5	20.8 ± 1.5	24.5 ± 2.0	55.1 ± 3.6
		III	82	29.2 ± 0.0	6.5 ± 0.1	29.9 ± 0.7	28.4 ± 0.2	41.7 ± 0.9
			96	29.6 ± 0.0	6.2 ± 0.1	25.3 ± 0.4	26.1 ± 0.3	48.5 ± 0.8
			103	29.4 ± 0.0	6.5 ± 0.1	27.0 ± 0.5	27.3 ± 0.3	45.6 ± 0.6

Heathland	*C. vulgaris*	I	0	31.3 ± 0.0	11.5 ± 0.2	4.2 ± 0.2	10.8 ± 0.6	85.0 ± 0.5
			12	31.4 ± 0.1	10.9 ± 0.5	3.7 ± 1.0	10.7 ± 0.7	85.5 ± 0.9
			27	31.5 ± 0.0	11.7 ± 0.2	3.6 ± 0.1	10.1 ± 0.2	86.3 ± 0.4
		II	40	31.3 ± 0.0	10.9 ± 0.2	4.9 ± 1.1	10.9 ± 0.5	84.1 ± 1.3
			54	31.1 ± 4.0	11.5 ± 0.3	6.3 ± 3.4	13.9 ± 4.4	79.8 ± 7.4
			68	31.6 ± 0.0	11.6 ± 0.1	2.7 ± 0.1	8.8 ± 0.2	88.4 ± 0.3
		III	82	31.5 ± 0.0	11.4 ± 0.2	3.5 ± 0.3	9.7 ± 0.2	86.8 ± 0.4
			96	31.2 ± 0.1	10.6 ± 1.0	4.2 ± 0.6	11.4 ± 0.9	84.3 ± 1.3
			103	31.4 ± 0.0	11.3 ± 0.5	3.8 ± 0.4	10.1 ± 0.4	86.0 ± 0.7
	Roots	I	0	32.0 ± 0.2	7.0 ± 0.9	21.6 ± 3.8	19.4 ± 2.1	58.9 ± 5.7
			12	30.3 ± 0.1	5.3 ± 0.9	16.4 ± 2.9	23.8 ± 4.5	59.8 ± 3.0
			27	29.7 ± 0.1	3.7 ± 0.3	26.9 ± 2.6	24.9 ± 2.4	48.1 ± 3.5
		II	40	29.3 ± 0.2	2.7 ± 0.3	27.4 ± 1.9	24.7 ± 1.3	44.2 ± 1.9
			54	30.7 ± 0.3	3.9 ± 0.7	31.1 ± 2.3	24.1 ± 2.5	44.1 ± 4.6
			68	29.7 ± 0.3	4.0 ± 0.4	27.7 ± 4.5	20.4 ± 2.3	51.9 ± 6.2
		III	82	29.5 ± 0.3	3.6 ± 0.5	32.7 ± 4.7	21.1 ± 3.5	47.2 ± 7.0
			96	29.9 ± 0.2	1.8 ± 0.3	36.0 ± 7.4	24.9 ± 4.6	39.0 ± 1.9
			103	29.9 ± 0.2	4.2± 0.2	25.9 ± 4.5	18.0 ± 1.8	56.1 ± 6.1
	Soils	I	0	29.0 ± 0.1	5.2 ± 0.4	36.4 ± 1.3	23.1 ± 3.6	40.4 ± 4.7
			12	30.2 ± 0.1	9.1 ± 0.1	19.5 ± 0.4	19.6 ± 0.8	60.9 ± 1.0
			27	30.2 ± 0.1	8.9 ± 0.7	19.7 ± 0.6	19.4 ± 1.3	60.9 ± 1.0
		II	40	30.6 ± 0.1	8.4 ± 0.1	13.7 ± 0.8	15.1 ± 0.6	71.2 ± 1.4
			54	30.5 ± 0.1	10.4 ± 0.2	15.6 ± 0.8	17.5 ± 1.2	66.9 ± 1.9
			68	30.1 ± 0.2	9.3 ± 0.2	19.5 ± 1.4	21.4 ± 1.6	59.1 ± 2.9
		III	82	29.8 ± 0.0	8.1 ± 0.2	22.5 ± 0.6	21.4 ± 0.5	56.0 ± 0.8
			96	30.3 ± 0.0	8.6 ± 0.3	18.5 ± 0.6	18.3 ± 0.5	63.1 ± 1.2
			103	30.0 ± 0.1	8.4 ± 0.1	21.4 ± 0.6	20.8 ± 0.6	57.7 ± 0.8

ACL, Average chain length; CPI, Carbon preference index

Supplementary data (Manuscript III)

Table S.4: $^{13}CO_2$ labelling experiment conducted in the field and samples collected in Summer 2011 in the EVENT I experiment Bayreuth, Germany

		Number of drought days	0	1	2	6	12	27	33	40	44	45	46	47	54	68	69	71	72	76	82	89	96	103	114	121	128
		Month	May 2011					June 2011				July 2011								August 2011					September 2011		
		Plot #, Date	18	19	20	24	30	14	20	27	30	1	2	4	11	25	26	27	28	1	8	15	22	28	9	16	23
Shoots	Grassland	10		×	×(OLP)	×	×	×	×	×		×		×	× ×	×		×		× ×	×	× ×	×	× ×	× ×	× ×	× ×
		27		×	×(OLP)	×	×	×	×	×		×		×	× ×	×		×		× ×	×	× ×	×	× ×	× ×	× ×	× ×
		69		×	×(OLP)	×	×	×	×	×		×		×	× ×	×		×		× ×	×	× ×	×	× ×	× ×	× ×	× ×
		75		×	×(OLP)	×	×	×	×	×		×		×	× ×	×		×		× ×	×	× ×	×	× ×	× ×	× ×	× ×
		113		×	×(OLP)	×	×	×	×	×		×		×	× ×	×		×		× ×	×	× ×	×	× ×	× ×	× ×	× ×
		118		×	×(OLP)	×	×	×	×	×		×		×	× ×	×		×		× ×	×	× ×	×	× ×	× ×	× ×	× ×
		44			×		×	×		×					×	×					×			×	×	×	×
		59			×		×	×		×					×	×					×			×	×	×	×
		125			×		×	×		×					×	×					×			×	×	×	×
		132			×		×	×		×					×	×					×			×	×	×	×
	Heathland	11			×(OLP)	×	×	×	×	×			×	×	× ×	×			×	× ×	×	× ×	×	× ×	× ×	× ×	× ×
		25			×(OLP)	×	×	×	×	×			×	×	× ×	×			×	× ×	×	× ×	×	× ×	× ×	× ×	× ×
		67			×(OLP)	×	×	×	×	×			×	×	× ×	×			×	× ×	×	× ×	×	× ×	× ×	× ×	× ×
		74			×(OLP)	×	×	×	×	×			×	×	× ×	×			×	× ×	×	× ×	×	× ×	× ×	× ×	× ×
		98			×(OLP)	×	×	×	×	×			×	×	× ×	×			×	× ×	×	× ×	×	× ×	× ×	× ×	× ×
		119			×(OLP)	×	×	×	×	×			×	×	× ×	×			×	× ×	×	× ×	×	× ×	× ×	× ×	× ×
		46			×		×	×		×					×	×					×			×	×	×	×
		60			×		×	×		×					×	×					×			×	×	×	×
		126			×		×	×		×					×	×					×			×	×	×	×
		141			×		×	×		×					×	×					×			×	×	×	×
Soil	Grassland	10			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		27			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		69			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		75			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		113			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		118			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		44			×		×	×		×					×	×					×			×	×	×	×
		59			×		×	×		×					×	×					×			×	×	×	×
		125			×		×	×		×					×	×					×			×	×	×	×
		132			×		×	×		×					×	×					×			×	×	×	×
	Heathland	11			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		25			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		67			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		74			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		98			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		119			×(OLP)		×	×		×				×	×	×				×	×	× ×	×	× ×	× ×	× ×	× ×
		46			×		×	×		×					×	×					×			×	×	×	×
		60			×		×	×		×					×	×					×			×	×	×	×
		126			×		×	×		×					×	×					×			×	×	×	×
		141			×		×	×		×					×	×					×			×	×	×	×

Labelled subplot area = 1 x 1 m^2, Each plot area = 2 x 2 m^2, Abbreviation used: OLP = Opposite area of the labelled plots = unlabelled

Community composition: Grassland (mixed plant culture: *Planatgo lanceolata* , *Holcus lanatus* , *Lotus corniculatus* , *Arrehenatherum elatius*) and Heathland (*Calluna vulgaris* and *Vaccinium myrtillus*)

First $^{13}CO_2$ labelling

Second $^{13}CO_2$ labelling

Third $^{13}CO_2$ labelling

Unlabelled plots

× Sample from first labelled plots

× Sample from second labelled plots

× Sample from third labelled plots

× Sample from unlabelled plots

Table S.5: Relative allocation of $^{13}C_{excess}$ (%) in roots vs. shoots in grassland and heathland communities after first, second and third $^{13}CO_2$ pulse-chase labelling that were applied during drought phase I, II and III respectively. These values are based on the approximation only since data of $^{13}C_{excess}$ (g m^{-2}) was available for only selected plants from the plots. The dash (-) indicates no roots sample were available on that day. Shaded area shows the results of irrigation treatment followed by drought.

				Grassland roots			Heathland roots		
Treatments	$^{13}CO_2$ labelling	Experiment (days)	Date of sampling	1st labelling	2nd labelling	3rd labelling	1st labelling	2nd labelling	3rd labelling
Control		0	18.05.2011	-			-		
Drought	Phase I	2	20.05.2011	-			-		
		6	24.05.2011	-			-		
		12	30.05.2011	7.91			0.96		
		27	14.06.2011	11.33			0.55		
		33	20.06.2011	-			-		
		40	27.06.2011	15.17			0.77		
	Phase II	44	01.07.2011	-	-		-	-	
		47	04.07.2011	-	7.97		-	0.21	
		54	11.07.2011	-	11.15		-	0.62	
		68	25.07.2011	-	14.84		-	0.77	
	Phase III	71	28.07.2011	-	-	-	-	-	-
		76	01.08.2011	-	-	-	-	-	-
		82	08.08.2011	-	-	2.79	-	-	1.60
		89	15.08.2011	-	6.89	1.73	-	0.56	0.80
		96	22.08.2011	-	-	3.43	-	-	2.76
		103	29.08.2011	-	6.12	3.66	-	0.30	5.81
Irrigation		114	09.09.2011	-	5.50	9.03	-	0.22	2.83
		121	16.09.2011	-	9.39	8.14	-	1.10	10.04
		128	23.09.2011	-	3.59	4.07	-	0.96	7.33

Table S.6: Relative allocation of $^{13}C_{excess}$ (%) in soil from plants in grassland and heathland communities after first, second and third $^{13}CO_2$ pulse-chase labelling that were applied during drought phase I, II and III respectively. These values are based on the approximation only since data of $^{13}C_{excess}$ (g m^{-2}) was available for only selected plants from the plots. The dash (-) indicates no roots sample were available on that day. Shaded area shows the results of irrigation treatment followed by drought.

				Grassland soil			Heathland soil		
Treatments	$^{13}CO_2$ labelling	Experiment (days)	Date of sampling	1st labelling	2nd labelling	3rd labelling	1st labelling	2nd labelling	3rd labelling
Control		0	18.05.2011	-			-		
Drought	Phase I	2	20.05.2011	-			-		
		6	24.05.2011	-			-		
		12	30.05.2011	0.16			0.01		
		27	14.06.2011	0.18			0.02		
		33	20.06.2011	-			-		
		40	27.06.2011	0.28			0.04		
	Phase II	44	01.07.2011	-	-		-	-	
		47	04.07.2011	-	0.08		-	0.01	
		54	11.07.2011	-	0.18		-	0.01	
		68	25.07.2011	-	0.12		-	0.03	
	Phase III	71	28.07.2011	-	-	-	-	-	-
		76	01.08.2011	-	-	-	-	-	-
		82	08.08.2011	-	-	0.19	-	-	0.46
		89	15.08.2011	-	0.32	0.21	-	0.02	0.31
		96	22.08.2011	-	-	0.24	-	-	0.21
		103	29.08.2011	-	0.14	0.15	-	0.02	0.27
Irrigation		114	09.09.2011	-	0.12	0.18	-	0.01	0.13
		121	16.09.2011	-	0.08	0.00	-	0.02	0.38
		128	23.09.2011	-	0.04	0.06	-	0.03	0.20

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Curriculum Vitae

Name	**Kavita Srivastava**
Date of Birth	**15th August 1980**

Academic position and research experience

05/2013 – 04/2017	**PhD Student,** Department of Geography, Physical Geography, Soil Science and Biogeochemistry, University of Zurich, Switzerland
04/2011 – 04/2013	**Research assistant,** University of Bayreuth, Germany

Work experience

01/2007 – 01/2008	**Principal,** Sai Public Primary School, Lucknow, India
07/2005 – 09/2006	**Botany lecturer**, Intermediate College, Lucknow, India
05/2005 –01/2006	**Guest lecturer** (part time) Botany, Govt. Degree College, Lucknow, India
04/2004 – 04/2005	**Biology teacher,** Higher secondary school (10+2), Lucknow, India

Education

08/2002 – 11/2004	**Master: University of Lucknow, India** Degree: Master of Science (Botany)
07/1998 – 02/2002	**Bachelor: University of Lucknow, India** Degree: Bachelor of Science (Botany Honours)

Skills

Six years of laboratory experience in plant and soil lipid analysis using Soxhlet extraction, Solid phase extraction (SPE), gas chromatography (GC-FID) and mass spectrometry (GC-MS). Analysis of biogeochemical elements (carbon, hydrogen and nitrogen) and their stable isotopic composition using elemental analyzer (EA), EA coupled to isotope ratio mass spectrometer (EA-C-IRMS) and compound specific isotope analysis using GC-IRMS.

Computer knowledge	Windows, Mac OS, MS Office	
Programming	R Studio, IBM SPSS, XLSTAT statistical software	
Application/Tools	Adobe illustrator, Photoshop	
Language	**English**	Very good in written and speaking
	German	Good knowledge (B1-level)
	Hindi	Mother tounge

Contributions at international conferences

Poster presentation, Biogeomon 2014: 8th International Symposium on Ecosystem Behaviour, Bayreuth, Germany

Oral presentation, General Assembly of the European Geosciences Union, April 2015, Vienna, Austria

Poster presentation, Plant Waxes: From Biosynthesis to Burial, June 2015 in Ascona, Switzerland

Poster presentation, International Meetings on Organic Geochemistry, September 2015, Prague, Czech Republic

Poster presentation, Joint European Stable Isotopes User group Meeting, September 2016, Ghent, Belgium

Poster presentation, American Geophysical Union, December 2016, San Francisco, USA

Research is creating new knowledge

Neil Armstrong